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<b>(21) International Application Number:</b> PCT/US98/11384 <b>(22) International Filing Date:</b> 1 June 1998 (01.06.98)  <b>(30) Priority Data:</b> 08/868,373 3 June 1997 (03.06.97) US  <b>(71) Applicant (for all designated States except US):</b> CARGILL, INCORPORATED [US/US]; 10547 West McGinty Road, Wayzata, MN 55391 (US).  <b>(71)(72) Applicants and Inventors:</b> JAWORSKI, Jan, G. [US/US]; 425 Emerald Woods Drive, Oxford, OH 45058 (US). POST-BEITTENMILLER, Martha, Ann [US/US]; 2375 Quail Road, Ardmore, OH 73491 (US). TODD, James [US/US]; 17 Kelly Drive, Oxford, OH 45056 (US).  <b>(74) Agent:</b> LUNDQUIST, Ronald, C.; Fish & Richardson P.C., P.A., Suite 3300, 60 South Sixth Street, Minneapolis, MN 55402 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> FATTY ACID ELONGASES  <b>(57) Abstract</b>  Nucleic acids are disclosed that encode fatty acid $\beta$ -keto acyl synthases from plants. Such synthases are effective for producing very long chain fatty acids (VLCFA), e.g., C22 to C26, preferentially saturated but also monounsaturated. Also disclosed are polypeptides encoded by such nucleic acids. Transgenic plants expressing these polypeptides exhibit altered levels of VLCFA in one or more tissues, such as seeds or leaves.		

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FATTY ACID ELONGASESField of the Invention

5        This invention relates to fatty acid elongase complexes and nucleic acids encoding elongase proteins. More particularly, the invention relates to nucleic acids encoding  $\beta$ -keto acyl synthase proteins that are effective for producing very long chain fatty acids, polypeptides  
10 produced from such nucleic acids and transgenic plants expressing such nucleic acids.

Background of the Invention

Plants are known to synthesize very long chain fatty acids (VLCFAs). VLCFAs are saturated or  
15 unsaturated monocarboxylic acids with an unbranched even-numbered carbon chain that is greater than 18 carbons in length. Many VLCFAs are 20-32 carbons in length, but VLCFAs can be up to 60 carbons in length. Important VLCFAs include erucic acid (22:1, i.e., a 22 carbon chain  
20 with one double bond), nervonic acid (24:1), behenic acid (22:0), and arachidic acid (20:0).

Plant seeds accumulate mostly 16- and 18-carbon fatty acids. VLCFAs are not desirable in edible oils. Oilseeds of the Crucifereae (e.g., rapeseed) and a few  
25 other plants, however, accumulate C20 and C22 fatty acids (FAs). Although plant breeders have developed rapeseed lines that have low levels of VLCFAs for edible oil purposes, even lower levels would be desirable. On the other hand, vegetable oils having elevated levels of  
30 VLCFAs are desirable for certain industrial uses, including uses as lubricants, fuels and as a feedstock for plastics, pharmaceuticals and cosmetics.

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The biosynthesis of saturated fatty acids up to an 18-carbon chain occurs in the chloroplast. C2 units from acyl thioesters are linked sequentially, beginning with the condensation of acetyl Coenzyme A (CoA) and malonyl acyl carrier protein (ACP) to form a C4 acyl fatty acid. This condensation reaction is catalyzed by a  $\beta$ -ketoacyl synthase III (KASIII).  $\beta$ -ketoacyl moieties are also referred to as 3-ketoacyl moieties.

The enzyme  $\beta$ -ketoacyl synthase I (KASI) is involved in the addition of C2 groups to form the C6 to C16 saturated fatty acids. KASI catalyzes the stepwise condensation of a fatty acyl moiety (C4 to C14) with malonyl-ACP to produce a 3-ketoacyl-ACP product that is 2 carbons longer than the substrate. The last condensation reaction in the chloroplast, converting C16 to C18, is catalyzed by  $\beta$ -ketoacyl synthase II (KASII).

Each elongation cycle involves three additional enzymatic steps in addition to the condensation reaction as discussed above. Briefly, the  $\beta$ -ketoacyl condensation product is reduced to  $\beta$ -hydroxyacyl-ACP, dehydrated to the enoyl-ACP, and finally reduced to a fully reduced acyl-ACP. The fully reduced fatty acyl-ACP reaction product then serves as the substrate for the next cycle of elongation.

The C18 saturated fatty acid (stearic acid, 18:0) can be transported out of the chloroplast and converted to the monounsaturate C18:1 (oleic acid), and the polyunsaturates C18:2 (linoleic acid) and C18:3 ( $\alpha$ -linolenic acid). C18:0 and C18:1 can also be elongated outside the chloroplast to form VLCFAs. The formation of VLCFAs involves the sequential condensation of two carbon groups from malonyl CoA with a C18:0 or C18:1 fatty acid substrate. Elongation of fatty acids longer than 18 carbons depends on the activity of a fatty acid elongase complex to carry out four separate enzyme reactions

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similar to those described above for fatty acid synthesis in the chloroplast. Fehling, Biochem. Biophys. Acta 1082:239-246 (1991). In plants, elongase complexes are distinct from fatty acid synthases since elongases are  
5 extraplastidial and membrane bound.

Mutations have been identified in an *Arabidopsis* gene associated with fatty acid elongation. This gene, designated the *FAE1* gene, is involved in the condensation step of an elongation cycle. See, WO 96/13582,  
10 incorporated herein by reference. Plants carrying a mutation in *FAE1* have significant decreases in the levels of VLCFAs in seeds. Genes associated with wax biosynthesis in jojoba have also been cloned and sequenced (WO 95/15387, incorporated herein by  
15 reference).

Very long chain fatty acids are key components of many biologically important compounds in animals, plants, and microorganisms. For example, in animals, the VLCFA arachidonic acid is a precursor to many prostaglandins.  
20 In plants VLCFAs are major constituents of triacylglycerols in many seed oils, are essential precursors for cuticular wax production, and are utilized in the synthesis of glycosylceramides, an important component of the plasma membrane.

25 Obtaining detailed information on the biochemistry of KAS enzymes has been hampered by the difficulties encountered when purifying membrane bound enzymes. Although elongase activities have been partially purified from a number of sources, or studied using cell  
30 fractions, the elucidation of the biochemistry of elongase complexes has been hampered by the complexity of the membrane fractions used as the enzyme source. For example, until recently, it was unclear as to whether plant elongase complexes were composed of a  
35 multifunctional polypeptide similar to the FAS found in

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animals and yeast, or if the complexes existed as discrete and dissociable enzymes similar to the FAS of plants and bacteria. Partial purification of an elongase KAS, immunoblot identification of the hydroxy acyl  
5 dehydrase, and the recent cloning of a KAS gene (*FAE1*) suggest that the enzyme activities of elongase complexes exist on individual enzymes.

#### Summary of the Invention

The invention disclosed herein relates to an  
10 isolated polynucleotide selected from one of the following: SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:5; SEQ ID NO:7; SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:13; an RNA analog of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, or 15; and a polynucleotide having a nucleic acid sequence  
15 complementary to one of the above. The polynucleotide can also be a nucleic acid fragment of one of the above sequences that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ  
20 ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14.

Also disclosed herein is an isolated polypeptide that has an amino acid sequence substantially identical to one of the following: SEQ ID NO:2, SEQ ID NO:4, SEQ ID  
25 NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14. Also disclosed are isolated polynucleotides encoding polypeptides substantially identical in their amino acid sequence to: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID  
30 NO:14.

The invention also relates to a transgenic plant containing a nucleic acid construct. The nucleic acid construct comprises a polynucleotide described above. The construct further comprises a regulatory element

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operably linked to the polynucleotide. The regulatory element may be a tissue-specific promoter, for example, an epidermal cell-specific promoter or a seed-specific promoter. The regulatory element may be operably linked to the polynucleotide in sense or antisense orientation. The plant has altered levels of very long chain fatty acids in tissues where the polynucleotide is expressed, compared to a parental plant lacking the nucleic acid construct.

10 A method is disclosed for altering the levels of very long chain fatty acids in a plant. The method comprises the steps of creating a nucleic acid construct and introducing the construct into the plant. The construct includes a polynucleotide selected from one of the following: SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:5; SEQ ID NO:7; SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:13; an RNA analog of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, or 15; and a polynucleotide having a nucleic acid sequence complementary to one of the above. The polynucleotide can also be a nucleic acid fragment of one of the above that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14. The polynucleotide is effective for altering the levels of very long chain fatty acids in the plant.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

#### 30 Brief Description of the Drawings

Figure 1 shows the time course of *in vitro* VLCFA synthesis by *FAE1* expressed in yeast, with 3 different acyl-CoA substrates.

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Figure 2 shows the rates of *in vitro* VLCFA synthesis and the VLCFA profiles of FAE1 expressed in yeast, with 3 different acyl-CoA substrates.

Figure 3 shows the nucleotide sequence of the  
5 coding region of the *Arabidopsis* EL1 polynucleotide (SEQ ID NO:1).

Figure 4 shows the deduced amino acid sequence (SEQ ID NO:2) for the EL1 coding sequence of Figure 3.

Figure 5 shows the nucleotide sequence of the  
10 coding region of the *Arabidopsis* EL2 polynucleotide (SEQ ID NO:3).

Figure 6 shows the deduced amino acid sequence (SEQ ID NO:4) for the EL2 coding sequence of Figure 5.

Figure 7 shows the nucleotide sequence of the  
15 coding region of the *Arabidopsis* EL3 polynucleotide (SEQ ID NO:5).

Figure 8 shows the deduced amino acid sequence (SEQ ID NO:6) for the EL3 coding sequence of Figure 7.

Figure 9 shows the nucleotide sequence of the  
20 coding region of the *Arabidopsis* EL4 polynucleotide (SEQ ID NO:7).

Figure 10 shows the deduced amino acid sequence (SEQ ID NO:8) for the EL4 coding sequence of Figure 9.

Figure 11 shows the nucleotide sequence of the  
25 coding region of the *Arabidopsis* EL5 polynucleotide (SEQ ID NO:9).

Figure 12 shows the deduced amino acid sequence (SEQ ID NO:10) for the EL5 coding sequence of Figure 11.

Figure 13 shows the nucleotide sequence of the  
30 coding region of the *Arabidopsis* EL6 polynucleotide (SEQ ID NO:11).

Figure 14 shows the deduced amino acid sequence (SEQ ID NO:12) for the EL6 coding sequence of Figure 13.



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Figure 15 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL7 polynucleotide (SEQ ID NO:13).

Figure 16 shows the deduced amino acid sequence  
5 (SEQ ID NO:14) for the EL7 coding sequence of Figure 15.

#### Description of the Preferred Embodiments

The present invention comprises isolated nucleic acids (polynucleotides) that encode polypeptides having  $\beta$ -ketoacyl synthase activity. The novel polynucleotides  
10 and polypeptides of the invention are involved in the synthesis of very long chain fatty acids and are useful for modulating the total amounts of such fatty acids and the specific VLCFA profile in plants.

A polynucleotide of the invention may be in the  
15 form of RNA or in the form of DNA, including cDNA, synthetic DNA or genomic DNA. The DNA may be double-stranded or single-stranded, and if single-stranded, can be either the coding strand or non-coding strand. An RNA analog may be, for example, mRNA or a combination of  
20 ribo- and deoxyribonucleotides. Illustrative examples of a polynucleotide of the invention are shown in Figs. 3, 5, 7, 9, 11, 13 and 15.

A polynucleotide of the invention typically is at least 15 nucleotides (or base pairs, bp) in length. In  
25 some embodiments, a polynucleotide is about 20 to 100 nucleotides in length, or about 100 to 500 nucleotides in length. In other embodiments, a polynucleotide is greater than about 1500 nucleotides in length and encodes a polypeptide having the amino acid sequence shown in  
30 Figs. 4, 6, 8, 10, 12, 14 or 16.

In some embodiments, a polynucleotide of the invention encodes analogs or derivatives of a polypeptide having the deduced amino acid sequence of Figs. 4, 6, 8,

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10, 12, 14 or 16. Such fragments, analogs or derivatives include, for example, naturally occurring allelic variants, non-naturally occurring allelic variants, deletion variants and insertion variants, that do not  
5 substantially alter the function of the polypeptide.

A polynucleotide of the invention may further comprise additional nucleic acids. For example, a nucleic acid fragment encoding a secretory or leading amino acid sequence can be fused in-frame to the amino  
10 terminal end of one of the EL1 through EL7 polypeptides. Other nucleic acid fragments are known in the art that encode amino acid sequences useful for fusing in-frame to the KAS polypeptides disclosed herein. See, e.g., U.S.  
5,629,193 incorporated herein by reference. A  
15 polynucleotide may further comprise one or more regulatory elements operably linked to a KAS polynucleotide disclosed herein.

The present invention also comprises polynucleotides that hybridize to a KAS polynucleotide  
20 disclosed herein. Such a polynucleotide typically is at least 15 nucleotides in length. Hybridization typically involves Southern analysis (Southern blotting), a method by which the presence of DNA sequences in a target nucleic acid mixture are identified by hybridization to a  
25 labeled oligonucleotide or DNA fragment probe. Southern analysis typically involves electrophoretic separation of DNA digests on agarose gels, denaturation of the DNA after electrophoretic separation, and transfer of the DNA to nitrocellulose, nylon, or another suitable membrane  
30 support for analysis with a radiolabeled, biotinylated, or enzyme-labeled probe as described in sections 9.37-9.52 of Sambrook et al., (1989) *Molecular Cloning*, second edition, Cold Spring Harbor Laboratory, Plainview, NY.

A polynucleotide can hybridize under moderate  
35 stringency conditions or, preferably, under high

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stringency conditions to a KAS polynucleotide disclosed herein. High stringency conditions are used to identify nucleic acids that have a high degree of homology to the probe. High stringency conditions can include the use of

5 low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate (0.1X SSC); 0.1% sodium lauryl sulfate (SDS) at 65°C. Alternatively, a denaturing agent such as formamide can be employed during hybridization, e.g., 50% formamide with 0.1%

10 bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is the use of 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium

15 phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS.

Moderate stringency conditions refers to

20 hybridization conditions used to identify nucleic acids that have a lower degree of identity to the probe than do nucleic acids identified under high stringency conditions. Moderate stringency conditions can include the use of higher ionic strength and/or lower

25 temperatures for washing of the hybridization membrane, compared to the ionic strength and temperatures used for high stringency hybridization. For example, a wash solution comprising 0.060 M NaCl/0.0060 M sodium citrate (4X SSC) and 0.1% sodium lauryl sulfate (SDS) can be used

30 at 50°C, with a last wash in 1X SSC, at 65°C. Alternatively, a hybridization wash in 1X SSC at 37°C can be used.

Hybridization can also be done by Northern analysis (Northern blotting), a method used to identify

35 RNAs that hybridize to a known probe such as an

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oligonucleotide, DNA fragment, cDNA or fragment thereof,  
or RNA fragment. The probe is labeled with a  
radioisotope such as  $^{32}\text{P}$ , by biotinylation or with an  
enzyme. The RNA to be analyzed can be usually  
5 electrophoretically separated on an agarose or  
polyacrylamide gel, transferred to nitrocellulose, nylon,  
or other suitable membrane, and hybridized with the  
probe, using standard techniques well known in the art  
such as those described in sections 7.39-7.52 of Sambrook  
10 et al., *supra*.

A polynucleotide has at least about 70% sequence  
identity, preferably at least about 80% sequence  
identity, more preferably at least about 90% sequence  
identity to SEQ ID NO:1, 3, 5, 7, 9, 11, or 13. Sequence  
15 identity can be determined, for example, by computer  
programs designed to perform single and multiple sequence  
alignments.

A polynucleotide of the invention can be obtained  
by chemical synthesis, isolation and cloning from plant  
20 genomic DNA or other means known to the art, including  
the use of PCR technology carried out using  
oligonucleotides corresponding to portions of SEQ ID  
NO:1, 3, 5, 7-9, 11 or 13. Polymerase chain reaction  
(PCR) refers to a procedure or technique in which target  
25 nucleic acid is amplified in a manner similar to that  
described in U.S. Patent No. 4,683,195, incorporated  
herein by reference, and subsequent modifications of the  
procedure described therein. Generally, sequence  
information from the ends of the region of interest or  
30 beyond is employed to design oligonucleotide primers that  
are identical or similar in sequence to opposite strands  
of the template to be amplified. PCR can be used to  
amplify specific RNA sequences, specific DNA sequences  
from total genomic DNA, and cDNA transcribed from total  
35 cellular RNA, bacteriophage or plasmid sequences, and the

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like. Alternately, a cDNA library (in an expression vector) can be screened with KAS-specific antibody prepared using peptide sequence(s) from hydrophilic regions of the KAS protein of SEQ ID NO:2 and technology  
5 known in the art.

A polypeptide of the invention comprises an isolated polypeptide having the deduced amino acid sequence of Figs. 2, 4, 6, 8, 10 and 12, as well as derivatives and analogs thereof. By "isolated" is meant  
10 a polypeptide that is expressed and produced in an environment other than the environment in which the polypeptide is naturally expressed and produced. For example, a plant polypeptide is isolated when expressed and produced in bacteria or fungi. Similarly, a plant  
15 polypeptide is isolated when its gene coding sequence is operably linked to a chimeric regulatory element and expressed in a tissue where the polypeptide is not naturally expressed. A polypeptide of the invention also comprises variants of the KAS polypeptides disclosed  
20 herein, as discussed above.

A full-length KAS coding sequence may comprise the sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11 or 13. Alternatively, a chimeric full-length KAS coding sequence may be formed by linking, in-frame, nucleotides from the  
25 5' region of a first KAS gene to nucleotides from the 3' region of a second KAS gene, thereby forming a chimeric KAS protein.

It should be appreciated that nucleic acid fragments having a nucleotide sequence other than the KAS  
30 sequences disclosed in SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 will encode a polypeptide having the exemplified amino acid coding sequence of SEQ ID NO:2, 4, 6, 8, 10, 12 or 14, respectively. The degeneracy of the genetic code is well-known to the art; i.e., for many amino acids, there

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is more than one nucleotide triplet which serves as the codon for the amino acid.

It should also be appreciated that certain amino acid substitutions can be made in protein sequences  
5 without affecting the function of the protein.

Generally, conservative amino acid substitutions or substitutions of similar amino acids are tolerated without affecting protein function. Similar amino acids can be those that are similar in size and/or charge  
10 properties, for example, aspartate and glutamate and isoleucine and valine are both pairs of similar amino acids. Similarity between amino acid pairs has been assessed in the art in a number of ways. For example, Dayhoff et al. (1978) in *Atlas of Protein Sequence and*  
15 *Structure*, Vol. 5, Suppl. 3, pp. 345-352, which is incorporated by reference herein, provides frequency tables for amino acid substitutions which can be employed as a measure of amino acid similarity.

A nucleic acid construct of the invention  
20 comprises a polynucleotide as disclosed herein linked to another, different polynucleotide. For example, a full-length KAS coding sequence may be operably fused in-frame to a nucleic acid fragment that encodes a leader sequence, secretory sequence or other additional amino  
25 acid sequences that may be usefully linked to a polypeptide or peptide fragment.

A transgenic plant of the invention contains a nucleic acid construct as described herein. In some embodiments, a transgenic plant contains a nucleic acid  
30 construct that comprises a polynucleotide of the invention operably linked to at least one suitable regulatory sequence in sense orientation. Regulatory sequences typically do not themselves code for a gene product. Instead, regulatory sequences affect the  
35 expression level of the polynucleotide to which they are

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linked. Examples of regulatory sequences are known in the art and include, without limitation, minimal promoters and promoters of genes preferentially or exclusively expressed in seeds or in epidermal cells of stems and leaves. Native regulatory sequences of the polynucleotides disclosed herein can be readily isolated by those skilled in the art and used in constructs of the invention. Other examples of suitable regulatory sequences include enhancers or enhancer-like elements, introns, 3' non-coding regions such as poly A sequences and other regulatory sequences discussed herein. Molecular biology techniques for preparing such chimeric genes are known in the art.

In other embodiments, a transgenic plant contains a nucleic acid construct comprising a partial or a full-length KAS coding sequence operably linked to at least one suitable regulatory sequence in antisense orientation. The chimeric gene can be introduced into a plant and transgenic progeny displaying expression of the antisense construct are identified.

One may use a polynucleotide disclosed herein for cosuppression as well as for antisense inhibition. Cosuppression of genes in plants may be achieved by expressing, in the sense orientation, the entire or partial coding sequence of a gene. See, e.g., WO 04/11516, incorporated herein by reference.

Transgenic techniques for use in the invention include, without limitation, *Agrobacterium*-mediated transformation, viral vector-mediated transformation, electroporation and particle gun transformation. Illustrative examples of transformation techniques are described in U.S. Patent 5,204,253, (particle gun) and U.S. Patent 5,188,958 (*Agrobacterium*), incorporated herein by reference. Transformation methods utilizing the Ti and Ri plasmids of *Agrobacterium* spp. typically

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use binary-type vectors. Walkerpeach, C. et al., in Plant Molecular Biology Manual, S. Gelvin and R. Schilperoort, eds., Kluwer Dordrecht, C1:1-19 (1994). If cell or tissue cultures are used as the recipient tissue for transformation, plants can be regenerated from transformed cultures by techniques known to those skilled in the art.

Techniques are known for the introduction of DNA into monocots as well as dicots, as are the techniques for culturing such plant tissues and regenerating those tissues. Monocots which have been successfully transformed and regenerated include wheat, corn, rye, rice, and asparagus. See, e.g., U.S. Patent Nos. 5,484,956 and 5,550,318, incorporated herein by reference.

For efficient production of transgenic plants from plant cells, it is desirable that the plant tissue used for transformation possess a high capacity for regeneration. Transgenic plants of woody species such as poplar and aspen have also been obtained. Technology is also available for the manipulation, transformation, and regeneration of gymnosperm plants. For example, U.S. Patent No. 5,122,466 describes the biolistic transformation of conifers, with preferred target tissue being meristematic and cotyledon and hypocotyl tissues. U.S. Patent No. 5,041,382 describes enrichment of conifer embryonal cells.

Seeds produced by a transgenic plant(s) can be grown and then selfed (or outcrossed and selfed) to obtain seeds homozygous for the construct. Seeds can be analyzed in order to identify those homozygotes having the desired expression of the construct. Transgenic plants may be entered into a breeding program, e.g., to introgress the novel construct into other lines, to transfer the construct to other species, or for further



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selection of other desirable traits. Alternatively, transgenic plants may be propagated vegetatively for those species amenable to such techniques. A nucleic acid construct of the invention can alter the levels of very long chain fatty acids in plant tissues expressing the polynucleotide, compared to VLCFA levels in corresponding tissues from an otherwise identical plant not expressing the polynucleotide. A comparison can be made, for example, between a non-transgenic plant of a plant line and a transgenic plant of the same plant line. Levels of VLCFAs having 20-32 carbons and/or levels of VLCFAs having 32-60 carbons can be altered in plants disclosed herein. Plants having an altered VLCFA composition may be identified by techniques known to the skilled artisan, e.g., thin layer chromatography or gas-liquid chromatography (GLC) analysis of the appropriate plant tissue.

A suitable group of plants with which to practice the invention are the *Brassica* species, including *B. napus*, *B. rapa*, *B. juncea*, and *B. hirta*. Other suitable plants include, without limitation, soybean (*Glycine max*), sunflower (*Helianthus annuus*) and corn (*Zea mays*).

A method according to the invention comprises introducing a nucleic acid construct into a plant cell and producing a plant (as well as progeny of such a plant) from the transformed cell. Progeny includes descendants of a particular plant or plant line, e.g., seeds developed on an instant plant are descendants. Progeny of an instant plant include seeds formed on  $F_1$ ,  $F_2$ ,  $F_3$ , and subsequent generation plants, or seeds formed on  $BC_1$ ,  $BC_2$ ,  $BC_3$ , and subsequent generation plants.

Methods and compositions according to the invention are useful in that the resulting plants and plant lines have desirable alterations in very long chain fatty acid composition. Suitable tissues in which to

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express polynucleotides and/or polypeptides of the invention include, without limitation, seeds, stems and leaves. Leaf tissues of interest include cells and tissues of the epidermis, e.g., cells that are involved  
5 in forming trichomes. Of particular interest are epidermal cells involved in forming the cuticular layer. The cuticular layer comprises various very long chain fatty acids and VLCFA derivatives such as alkanes, esters, alcohols and aldehydes. Altering the composition  
10 and amount of VLCFAs in epidermal cells and tissues may enhance defense mechanisms and drought tolerance of plants disclosed herein.

Polynucleotides of the invention can be used as markers in plant genetic mapping and plant breeding  
15 programs. Such markers may include RFLP, RAPD, or PCR markers, for example. Marker-assisted breeding techniques may be used to identify and follow a desired fatty acid composition during the breeding process. Marker-assisted breeding techniques may be used in  
20 addition to, or as an alternative to, other sorts of identification techniques. An example of marker-assisted breeding is the use of PCR primers that specifically amplify a sequence from a desired KAS that has been introduced into a plant line and is being crossed into  
25 other plant lines.

Plants and plant lines disclosed herein preferably have superior agronomic properties. Superior agronomic characteristics include, for example, increased seed germination percentage, increased seedling vigor,  
30 increased resistance to seedling fungal diseases (damping off, root rot and the like), increased yield, and improved standability.

While the invention is susceptible to various modifications and alternative forms, certain specific  
35 embodiments thereof are described in the general methods

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and examples set forth below. It should be understood, however, that these examples are not intended to limit the invention to the particular forms disclosed but, instead the invention is to cover all modifications, 5 equivalents and alternatives falling within the scope of the invention.

#### EXAMPLES

##### Example 1

##### **Cloning and Expression of FAE1 in Yeast Cells**

10       The open reading frame of the *Arabidopsis* *FAE1* gene was amplified directly by PCR, using *Arabidopsis thaliana* cv. Columbia genomic DNA as a template, pfu DNA polymerase and the following primers:

5'CTCGAGGAGCAATGACGTCCGTAA-3' and 5'-

15 CTCGAGTTAGGACCGACCGTTTGTG-3'. The PCR product was blunt-end cloned into the *Eco* RV site of pBluescript (Stratagene, La Jolla, CA),

      The *FAE1* gene was excised from the Bluescript vector with *Bam*HI, and then subcloned into the pYEura3 20 (Clontech, Palo Alto, CA). pYEura3 is a yeast centromere-containing, episomal plasmid that is propagated stably through cell division. The *FAE1* gene was inserted downstream of a *GAL1* promoter in pYEura3. The *GAL1* promoter is induced when galactose is present in 25 the medium and repressed when glucose is present in the growth medium.

      Insertion of the *FAE1* gene in the sense orientation was confirmed by PCR, and pYEura3/*FAE1* was used to transform *Saccharomyces cerevisiae* strain AB1380 30 using a lithium acetate procedure as described in Gietz, R. and Woods, R., in *Molecular Genetics of Yeast: Practical Approaches*, Oxford Press, pp. 121-134 (1994). Plasmid DNA was isolated from putative transformants, and

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the presence of the *FAE1*/pYEura3 construct was confirmed by Southern analysis.

Yeast transformed with pYEura3 having *FAE1* operably linked to the *GAL1* promoter were grown in the presence of galactose or glucose and were analyzed for the expression of *FAE1*. As a control, yeast transformed with pYEura3 containing no insert were also assayed. Analysis of such control preparations yielded fatty acid compositions and fatty acid elongation rates similar to those of yeast transformed with pYEura3/*FAE1* and grown with glucose as the carbon source.

The fatty acid composition of yeast cells grown in the presence of galactose was compared to that of cells grown in the presence of glucose, to determine if VLCFA were found in the galactose-induced cells.

Transformed yeast cells were grown overnight in YPD media at 30°C with vigorous shaking. One hundred  $\mu$ l of the overnight culture were used to inoculate 40 ml of complete minimal uracil dropout media (CM-Ura) supplemented with either glucose or galactose (2% w/v). Cultures were grown at 30°C to an OD<sub>600</sub> of approximately 1.3 to 1.5. Cells were harvested by centrifugation at 5000 Xg for 10 min. Total lipids were extracted from the cells with 2 volumes of 4N KOH in 100% methanol for 60 min. at 80°C. Fatty acids were saponified and methyl esters were prepared by drying the samples and resuspending in 0.5 ml of boron trichloride in methanol (10% v/v). Samples were incubated at 50°C for 15 min in a sealed tube. About 2 ml of water was then added and the fatty methyl esters were extracted thrice with 1 ml of hexane. Extracts were dried under nitrogen and redissolved in hexane. See Hlousek-Radojcic, A. et al., Plant J. 8:803-809. Methyl esters were analyzed on an HP 5890 series II gas chromatograph equipped with a 5771MSD and 7673 auto injector (Hewlett-Packard, Cincinnati, OH).

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Methyl esters were separated on a DB-23 (J&W Scientific) capillary column (30 m X 0.25 mm X 0.25  $\mu$ m). The column was operated with helium carrier gas and splitless injection (injection temperature 280°C, detector temperature 280°C). After an initial 3 min. at 100°C, the oven temperature was raised to 250° at 20°C min<sup>-1</sup> and maintained at that temperature for an additional 3 min. The identity of the peaks was verified by cochromatography with authentic standards and by mass spectrometer analysis.

The results clearly revealed the appearance of both 20:1 and 22:1 acyl-CoA products in galactose-induced yeast containing the *FAE1* coding sequence. Uninduced yeast cells failed to accumulated significant amounts of fatty acids longer than C18. These results indicate that expression of *FAE1* in yeast resulted in functional KAS activity and functional elongase activity.

#### Example 2

##### ***FAE1* Activity in Yeast Microsomes**

The functional expression of the *FAE1* KAS was analyzed by isolating microsomes from transformed yeast cells and assaying these microsomes *in vitro* for elongase activity.

Transformed yeast cells were grown in the presence of either glucose or galactose (2% w/v) as described in Example 1. Cells were harvested by centrifugation at 5000 Xg for 10 min and washed with 10 ml ice cold isolation buffer (IB), which contains 80 mM Hepes-KOH, pH 7.2, 5 mM EGTA, 5 mM EDTA, 10 mM KCl, 320 mM sucrose and 2 mM DTT). Cells were then resuspended in enough IB to fill a 1.7 ml tube containing 700  $\mu$ l of 0.5  $\mu$ m glass beads and yeast microsomes were isolated from the cells essentially as described in Tillman, T. and Bell, R., J. Biol. Chem. 261:9144-9149 (1986). The microsomal

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membrane pellet was recovered by centrifugation at 252,000 xg for 60 min. The pellet was rinsed by resuspending in 40 ml fresh IB and again recovered by centrifugation at 252000 Xg for 60 min. Microsomal  
5 pellets were resuspended in a minimal volume of IB, and the protein concentration adjusted to  $2.5 \mu\text{g } \mu\text{l}^{-1}$  by addition of IB containing 15% glycerol. Microsomes were frozen on dry ice and stored at  $-80^{\circ}\text{C}$ . The protein concentration in microsomes was determined by the  
10 Bradford method (Bradford, 1976).

Fatty acid elongase activity was measured essentially as described in Hlousek-Radojcic, A. et al., Plant J. 8:803-809 (1995). Briefly, the standard elongation reaction mix contained 80 mM Hepes-KOH, pH  
15 7.2, 20 mM  $\text{MgCl}_2$ , 500  $\mu\text{M}$  NADPH, 1 mM ATP, 100  $\mu\text{M}$  malonyl-CoA, 10  $\mu\text{M}$  CoA-SH and 15  $\mu\text{M}$  radioactive acyl-CoA substrate. The radiolabeled substrate was either  $[1\text{-}^{14}\text{C}]18:1\text{-CoA}$  (50 uCi  $\mu\text{mol}^{-1}$ ),  $[1\text{-}^{14}\text{C}]18:0\text{-CoA}$  (55 uCi  $\mu\text{mol}^{-1}$ ), or  $[1\text{-}^{14}\text{C}]16:0\text{-CoA}$  (54 uCi  $\mu\text{mol}^{-1}$ ). The reaction was  
20 initiated by the addition of yeast microsomes (5  $\mu\text{g}$  protein) and the mixture incubated at  $30^{\circ}\text{C}$  for the indicated period of time. The final reaction volume was 25  $\mu\text{l}$ .

Methyl esters of the acyl-CoA elongation products  
25 were prepared as described in Example 1. Methyl esters were separated on reversed phase silica gel KC18 TLC plates (Whatman, 250  $\mu\text{m}$  thick), quantified by phosphorimaging, and analyzed on by ImageQuant software (Molecular Dynamics, Inc., Sunnyvale, CA). The detection  
30 limit for each product is about 0.001 nanomoles per min. per mg microsomal protein, depending on the phosphorimage exposure time.

Results of representative in vitro elongation assays are shown in Figs. 1 and 2. The results indicate  
35 that microsomes from galactose-induced cells expressing

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*FAE1* catalyzed multiple cycles of elongation starting with either C16:0 acyl CoA, C18:0 acyl CoA, or C18:1 acyl-CoA as the substrate (Fig. 1). The 16:0 and 18:0 acyl-CoA substrates were elongated to C26:0 acyl-CoA. In contrast, the 18:1-CoA substrate was elongated primarily to C20:1, with only low levels of C22:1 acyl-CoA being produced. Occasionally, trace levels of C24:1 CoA were also observed. Although the chain length of the products from the 18:1 acyl-CoA substrate were less than the chain length from the saturated acyl-CoA substrates, the rate of elongation of oleoyl-CoA was about 2- and 3-fold higher than the rates of elongation of 16:0-CoA and 18:0-CoA, respectively.

The elongation activity observed in microsomes from uninduced cells indicated that there was a low level of endogenous elongase activity when 18:1-CoA or 18:0-CoA were used as substrates. There was substantial 16:0-CoA elongase activity (10.1 nmol mg protein<sup>-1</sup> at 30 min) in microsomes from uninduced cells (Fig. 2). However, the major product of 16:0 elongation using uninduced microsomes was C18:0 acyl CoA, with only small amounts of products beyond this length. The elongation of the 16:0 acyl-CoA substrate presumably is due to an endogenous yeast elongase.

Elongation of 18:1 CoA by microsomes from induced cells occurred at a rate about 18-fold higher than in microsomes isolated from the uninduced cells (Fig. 2). With microsomes from induced yeast, synthesis of 20:0 CoA from the 16:0 CoA substrate, occurred at a rate similar to that seen when the substrate was 18:0 CoA (4.2 vs. 5.1 nmol mg protein<sup>-1</sup>). The total rate of elongation of [<sup>14</sup>C] 16:0-CoA by microsomes from induced cells (15.8 nmol mg protein<sup>-1</sup> at 30 min.) was more than 50% higher than elongation of [<sup>14</sup>C] 16:0-CoA by microsomes from uninduced cells, suggesting that the *FAE1* KAS utilized 16:0-CoA as

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a substrate in addition to C18-C24 acyl-CoAs. The *FAE1* elongase KAS activity, i.e., the difference in the 16:0 elongation rates between microsomes from induced and uninduced cells, was 5.7 nmol mg protein<sup>-1</sup>. The  
5 elongation rate with the 16:0 substrate thus was similar to the elongase activity of the *FAE1* elongase KAS with the 18:0 substrate.

These results indicate that *FAE1* KAS expressed in yeast could synthesize 3-ketoacyl-CoA *in vitro* and, in  
10 combination with yeast reductases and dehydrases, could form a functional VLCFA elongase complex. In addition, these results suggest that *FAE1* is membrane-bound in yeast cells.

### Example 3

#### 15     **Cloning and Sequencing of *Arabidopsis* Elongase Genes**

The sequence of a jojoba seed cDNA (see WO 93/10241 and WO 95/15387, incorporated herein by reference) was used to search the *Arabidopsis* expressed sequence tag (EST) database of the *Arabidopsis* Genome  
20 Stock Center (The Ohio State University, Columbus, Ohio). The BLAST computer program (National Institutes of Health, Bethesda, MD, USA) was used to perform the search. The search identified two ESTs (ATTS1282 and ATTS3218) that had a high degree of sequence identity  
25 with the jojoba sequence. The ATTS1282 and ATTS3218 ESTs appeared to be partial cDNA clones rather than full-length clones based on the length of the jojoba sequence.

A genomic DNA library from *Arabidopsis thaliana* cv. Columbia, was prepared in the lambda GEM11 vector  
30 (Promega, Madison, Wisconsin) and was obtained from Ron Davis, Stanford University, Stanford, CA. The library was hybridized with ATTS1282 and ATTS3218 as probes and 2 clones were identified for each EST. Phage DNA was isolated from each of the hybridizing clones, the genomic



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insert was excised with the restriction enzyme Sac I and subcloned into the plasmid pBluescript (Stratagene, La Jolla, CA). One clone from the ATTS1282 hybridization was designated EL1 and one clone from the ATTS3218

5 hybridization was designated EL2.

A yeast expression library, containing cDNA from *Arabidopsis thaliana* cv. Columbia, was prepared in the lambda YES expression vector described in Elledge et al. (Elledge, S. et al., Proc. Natl. Acad. Sci USA 88:1731-10 1735 (1991) and was obtained from Ron Davis at Stanford University, Stanford, CA. The library was hybridized with a EL2 partial cDNA probe. A full-length EL2 cDNA was not identified. However, the probe did identify a full-length cDNA which was designated EL3.

15 A consensus sequence for the C-terminal region of EL1, EL2 and the jojoba cDNA polypeptides was identified by sequence alignment using DNA analysis programs from DNASTar, Madison, Wisconsin. This consensus sequence was used to search the *Arabidopsis* EST database again for  $\beta$ -20 keto acyl synthase sequences. These searches identified four additional putative  $\beta$ -keto acyl synthase ESTs, which were designated EL4 through EL7. EL4, EL5, EL6, and EL7 have homology to Genbank Accession Nos. T04345, T44939, T22193 and T76700, respectively.

25 The lambda YES cDNA expression library described above was hybridized with the EL1 and EL4-EL7 ESTs as probes. This screen identified full-length cDNAs for EL1, EL5 and EL6.

The lambda GEM11 genomic library was hybridized 30 with the EL4 and EL7 ESTs as probes. This screen identified full-length genomic clones for EL4 and EL7. Phage DNA was isolated from each of the hybridizing clones and subcloned into pBluescript as described above.

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The 7 EL clones were sequenced on both strands with regions of overlap for each sequence run.

Sequencing was carried out with an ABI automated sequencer (Applied Biosystems, Inc., Foster City, California), following the manufacturer's instructions.

The nucleotide sequences for the coding regions of EL1-EL7 are shown in Figs. 3, 5, 7, 9, 11, 13 and 15, respectively. The deduced amino acid sequences for EL1-EL7 are shown in Figs. 4, 6, 8, 10, 12, 14 and 16, respectively, using the standard one-letter amino acid code. The EL1, EL2 and EL7 genomic clones appeared to lack introns. The EL4 genomic clone contained one intron near the 5' end of the coding region.

The nucleotide sequences of the 7 EL polynucleotides were compared to 5 DNA sequences present in Genbank. Genbank, National Center for Biotechnology Information, Bethesda, MD. Two of the 5 accessions were cloned from members of the Brassicaceae: the *Arabidopsis* FAE1 sequence (Accession U29142) and a *Brassica napus* sequence (Accession U50771). Three of the accessions were cloned from jojoba (*Simmondsia chinensis*): 2 wax biosynthesis genes (Accessions I14084 and I14085) and a jojoba KAS gene (Accession U37088). See also U.S. Patent 5,445,947, incorporated herein by reference.

Multiple alignment of the 12 sequences was carried out with a computer program sold under the trade name MEGALIGN Lasergene by DNASTar (Madison, Wisconsin). Alignments were done using the Clustal method with weighted residue weight table. The nucleotide sequence similarity index and percent divergence based on the multiple alignment algorithm is shown in Table 1. The nucleotide sequences of EL1-EL7 are distinguishable from the 5 DNA sequences obtained from Genbank.

The deduced amino acid sequences of the EL1-7 polypeptides were compared with the MEGALIGN program to

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the deduced amino acid sequences of the same 5 Genbank clones, using the Clustal method with PAM250 residue weight table. The amino acid sequence similarity and percent divergence are shown in Table 2. The amino acid  
5 sequences of EL1-EL7 polypeptides are distinguishable from those of the Genbank sequences.

TABLE 1

Nucleotide sequence pair distances of EL1-EL7, using Clustal method with weighted residue weight table.

	1	2	3	4	5	6	7	8	9	10	11	12	
1		77.5	62.4	58.8	57.0	54.9	47.0	42.8	42.9	43.1	44.7	41.3	1 ARAPAE1 U29142
2	18.1		61.0	57.9	55.4	53.7	46.9	42.7	44.1	42.9	42.3	40.5	2 BNAFAE1 U50771
3	40.4	41.0		70.5	59.3	56.4	46.7	48.5	48.1	48.6	46.5	43.5	3 EL2
4	43.9	44.3	28.0		56.3	55.4	46.5	47.0	45.1	47.2	47.4	42.3	4 EL3
5	40.7	42.3	45.0	45.0		68.0	54.0	46.8	46.6	46.4	49.0	47.2	5 EL5
6	45.8	48.9	46.0	47.3	32.4		53.6	48.6	48.2	48.6	49.0	49.2	6 EL7
7	74.1	71.0	69.4	67.3	64.3	64.5		49.8	49.2	49.8	47.7	48.2	7 EL6
8	68.1	66.2	63.4	63.1	65.5	64.2	56.1		97.7	99.7	48.4	45.8	8 JOJOKCS U37088
9	67.0	65.4	63.7	64.6	64.6	64.1	56.6	1.1		95.9	47.6	44.8	9 JOKCS10 I14084
10	67.2	65.2	61.8	61.4	64.1	63.0	56.3	0.2	1.1		48.4	45.3	10 JOKCS11 I14085
11	88.6	85.8	81.0	77.0	77.4	82.4	83.1	71.1	71.1	69.9		48.3	11 EL1
12	95.7	90.4	95.4	91.5	84.5	82.8	91.9	73.4	73.8	73.3	59.9		12 EL4
	1	2	3	4	5	6	7	8	9	10	11	12	

**TABLE 2**  
Amino acid sequence pair distances of EL1-EL7, using Clustal  
method with PAM250 residue weight table.

	1	2	3	4	5	6	7	8	9	10	11	12	
1		72.0	62.9	59.8	60.9	60.2	50.3	51.9	52.1	51.5	49.1	42.0	1 EL2
2	31.1		60.1	57.5	58.7	57.1	49.8	49.8	50.0	49.2	49.6	44.4	2 EL3
3	47.4	48.7		82.4	60.7	63.0	50.0	51.4	51.6	50.8	47.8	43.9	3 ATFAE1 U29142
4	51.8	52.8	17.9		60.2	61.0	49.2	50.3	50.5	49.7	46.5	42.4	4 BNFAE1 U50771
5	49.0	51.3	45.8	46.2		75.8	61.0	58.7	58.9	58.3	55.0	55.6	5 EL7
6	52.6	55.5	42.8	46.5	29.3		61.8	55.7	55.7	54.9	52.9	50.5	6 EL5
7	74.7	70.5	71.8	74.4	52.0	50.8		52.8	52.8	51.8	53.4	51.6	7 EL6
8	66.7	69.2	66.2	67.3	54.8	59.8	67.7		99.8	96.9	53.1	52.0	8 JOJKCS U37088
9	66.3	68.7	66.2	67.3	54.0	59.3	67.7	0.2		96.9	53.1	51.9	9 JKCS11 I14085
10	66.3	69.7	66.6	67.8	54.5	60.7	68.6	1.8	1.6		51.7	50.7	10 JKCS10 I14084
11	73.6	73.7	72.8	74.4	60.8	66.0	67.2	63.9	63.9	65.3		50.8	11 EL1
12	84.8	85.5	82.7	83.3	60.6	70.8	67.1	68.5	68.5	69.9	69.4		12 EL4
	1	2	3	4	5	6	7	8	9	10	11	12	

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Example 4**Expression of EL1 and EL2 in Yeast**

The open reading frames (ORFs) for the EL2, EL4 and EL7 clones were amplified by PCR. The EL2 ORF was cloned into  $\lambda$ YES using the primers:

CTCGAGCAAGTCCACTACCAACGCA and CTCGAGCGAGTCAGAAGGAACAAA.

The EL4 ORF was cloned into pYEura3 using the primers: GATAATTTAGAGAGGCACAGGGT and GTCGACACAAGAATGGGTAGATCCAA.

The EL7 ORF was cloned into pYEura3 using the primers: CAGTTCCTCAAACGAAGCTA and GTCGACTTCTCAATGGACGGTGCCGGA.

Amplified products were cloned into pYEura3 under the control of, and 3' to, the GAL1 promoter. The resulting plasmids were transformed into yeast as described in Example 1.

Yeast cultures containing full-length EL1 in  $\lambda$ YES and full-length EL2 in pYEura3 were grown in the presence of galactose or glucose as described in Example 2. Microsomes were then prepared from each of the cultures and fatty acid elongation assays were carried out as described in Example 2.

In the first experiment, microsomes were prepared from galactose-induced cultures of EL1, EL2 and FAE1, and incubated with either [1- $^{14}$ C] 18:0 acyl-CoA or [1- $^{14}$ C] 18:1 acyl-CoA as substrate. The amounts of various reaction products synthesized after 30 minutes (min) were determined as described in Example 2. The results when 18:0 acyl-CoA was the substrate are shown in Table 3. The results when 18:1 acyl-CoA was the substrate are shown in Table 4.

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Table 3.  
Elongation of 18:0-CoA by FAE1, EL1 and EL2 Genes  
Expressed in Yeast

Acyl-CoA Product	β-Keto Acyl Synthase Gene					
	FAE1		EL1		EL2	
	Rate <sup>1</sup>	(%)	Rate	(%)	Rate	(%)
20:0	0.369	64.3	0.084	38.8	0.108	41.8
22:0	0.113	18.6	0.047	21.9	0.053	20.7
24:0	0.065	10.7	0.043	19.9	0.052	20.3
26:0	0.038	6.3	0.042	19.4	0.044	17.2
Total	0.585	100.0	0.216	100.0	0.258	100.0

<sup>1</sup> Nanomoles/minute/mg of microsomal protein

Table 4.  
Elongation of 18:1-CoA by FAE1, EL1 and EL2 Genes  
Expressed in Yeast

Acyl-CoA Product	β-Keto Acyl Synthase Gene					
	FAE1		EL1		EL2	
	Rate <sup>1</sup>	(%)	Rate	(%)	Rate	(%)
20:0	1.131	84.6	0.111	80.8	0.091	84.1
22:1	0.206	15.4	0.026	19.2	0.017	15.9
24:1	0.0	0.0	0.0	0.0	0.0	0.0
26:1	0.0	0.0	0.0	0.0	0.0	0.0
Total	1.337	100.0	0.137	100.0	0.108	100.0

<sup>1</sup> Nanomoles/minute/mg of microsomal protein

The results shown in Tables 3 and 4 indicate that the EL1 and EL2 gene products have β-ketoacyl synthase (KAS) activity and that the KAS reaction product can be utilized to form VLCFAs. The specific activities of the 3 KAS enzymes cannot be compared, since the relative amount of the heterologous KAS protein in each microsomal preparation is not known. However, the proportions of various reaction products can be compared between FAE1, EL1 and EL2.

The data shown in Table 3 indicate that the EL1 and EL2 KAS activities result in a higher proportion of saturated VLCFAs than does the FAE1 KAS activity. These

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results suggest that EL1 and EL2 encode novel gene products, because EL1 and EL2 have a greater preference for C22:0 and C24:0 acyl-CoA substrates than does *FAE1*.

A comparison of the relative elongation activity of *FAE1* with 18:0 and 18:1 substrates (Tables 3 and 4) indicates that *FAE1* is more active when 18:1 is the substrate than when 18:0 is the substrate. In contrast, the overall rate of product formation with EL1 is less when 18:1 is the substrate than when 18:0 is the substrate (Tables 3 and 4). EL2 is also less active when 18:1 is the substrate than when 18:0 is the substrate (Tables 3 and 4). These results support the conclusion that EL1 and EL2 encode novel gene products and suggest that EL1 and EL2 have a preference for saturated fatty acids as substrates, whereas the *FAE1* gene product has a preference for monounsaturated fatty acids as substrates.

In a second experiment, microsomes were prepared from galactose-induced and from glucose-repressed yeast cultures containing EL1 or EL2 coding sequences. The microsomal preparations were incubated with either 18:0 acyl-CoA or 18:1 acyl-CoA as substrate and the fatty acid reaction products determined as described above. The results with the 18:0 substrate are shown in Table 5. The results with the 18:1 substrate are shown in Table 6.



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Table 5.  
Elongation of 18:0-CoA by EL1 and EL2  
With and Without Induction of Gene Expression

Acyl CoA	E-Keto Acyl Synthase Gene							
	EL1				EL2			
	+Glucose		+Galactose		+Glucose		+Galactose	
	Rate <sup>1</sup>	(%)	Rate	(%)	Rate	(%)	Rate	(%)
20:0	0.007	100.0	0.074	55.8	0.030	81.3	0.107	43.1
22:0	0.000	0.0	0.023	17.4	0.002	5.1	0.044	17.8
24:0	0.000	0.0	0.020	15.3	0.005	13.6	0.048	19.1
26:0	0.000	0.0	0.015	11.5	0.000	0.0	0.050	20.0
Total	0.007	100.0	0.133	100.0	0.037	100.0	0.249	100.0

Nanomoles/minute/mg of microsomal protein

Table 6.  
Elongation of 18:1-CoA by EL1 and EL2  
With and Without Induction of Gene Expression

Acyl CoA	E-Keto Acyl Synthase Gene							
	EL1				EL2			
	+Glucose		+Galactose		+Glucose		+Galactose	
	Rate <sup>1</sup>	(%)	Rate	(%)	Rate	(%)	Rate	(%)
20:1	0.062	100.0	0.081	100.0	0.043	100.0	0.089	100.0
22:1	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0
24:1	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0
26:1	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0
Total	0.062	100.0	0.081	100.0	0.043	100.0	0.089	100.0

Nanomoles/minute/mg of microsomal protein

The results in Table 5 show *in vitro* elongase activity for EL1 and EL2 under induced (galactose) and uninduced (glucose) conditions. The comparison indicates that induction with galactose results in a large increase in overall elongase activity when 18:0 acyl CoA is the substrate (about 19-fold and 7-fold for EL1 and EL2, respectively). In contrast, induction when 18:1 acyl CoA is the substrate results in only a small increase in elongase activity (about 1.3-fold and 2-fold for EL1 and EL2, respectively), as shown in Table 6.

The results in Table 5 show that little or no VLCFA products are made by yeast microsomes under uninduced conditions. Upon induction of EL1 and EL2 gene

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expression, however, significant quantities of C20:0, C22:0, C24:0 and C26:0 are made. The data in Tables 5 and 6 are consistent with the results in Tables 3 and 4, which indicated that EL1 and EL2 were more active with a saturated fatty acid substrate than with a monounsaturated substrate.

The data in Tables 5 and 6 are also consistent with the data in Tables 3 and 4 indicating that the EL1 and EL2 gene products are more active in converting C24:0 to C26:0 than is FAE1.

In a third experiment, microsomes from induced and uninduced cultures containing EL1 or EL2 were incubated in the absence of cofactors involved in the  $\beta$ -ketoacyl condensation reaction. Cultures were induced and microsomes were prepared as described in Example 2. *In vitro* assays were carried out as described in Example 2, except that either ATP, CoASH or both were omitted from the enzyme reaction mixture. In addition, one reaction was carried out in a complete mixture having 0.01 mM of cerulenin (Sigma, St. Louis, MO). Cerulenin is an inhibitor of some condensing enzymes. The results are shown in Tables 7-9.

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Table 7.  
Effect of Cofactors on 18:0-CoA Elongation<sup>1</sup>

Gene	Expt <sup>4</sup>	+Glu <sup>2</sup>	+Gal <sup>2</sup>	-ATP <sup>3</sup>	-CoA <sup>3</sup>	-A&C <sup>3</sup>	+ Cer <sup>3</sup>
EL1	1	.037	.109	.095	.105	.119	.141
	2	N.D.	.090	.125	.093	.270	.176
EL2	1	.033	.112	.168	.127	.143	.238
	2	N.D.	.120	.178	.133	.195	.302

<sup>1</sup> Activity in nanomoles/minute/mg of microsomal protein.

<sup>2</sup> +Glu: microsomes from cultures grown in the presence of glucose and incubated in standard reaction mix; +Gal: microsomes from cultures grown in the presence of galactose and incubated in standard reaction mix.

<sup>3</sup> Microsomes from galactose-induced cultures. -ATP: ATP omitted from reaction mix; -CoA: Coenzyme A omitted from reaction mix; -A&C: ATP and Coenzyme A omitted from reaction mix; +Cer: Standard reaction mix containing 0.01 mM cerulenin.

<sup>4</sup> Experiment No.

Table 8.  
Effect of Cofactors on Elongation Products of EL1<sup>1</sup>

Prod.	+Glu <sup>2</sup>	+Gal <sup>2</sup>	-ATP <sup>3</sup>	-CoA <sup>3</sup>	-A&C <sup>3</sup>	+Cer <sup>3</sup>
20:0	53.9	46.2	34.4	47.8	41.7	46.7
22:0	14.4	18.7	13.7	18.0	19.4	16.2
24:0	18.5	18.1	20.6	19.1	16.7	17.7
26:0	13.2	17.1	31.4	15.2	22.3	19.4
Total	100.0	100.0	100.0	100.0	100.0	100.0

<sup>1</sup> Amount of indicated product as a percent of total products formed. Results of one experiment for +Glucose; Average of two experiments for other conditions.

<sup>2</sup> +Glu: microsomes from cultures grown in the presence of glucose and incubated in standard reaction mix; +Gal: microsomes from cultures grown in the presence of galactose and incubated in standard reaction mix.

<sup>3</sup> Microsomes from galactose-induced cultures. -ATP: ATP omitted from reaction mix; -CoA: Coenzyme A omitted from reaction mix; -A&C: ATP and Coenzyme A omitted from reaction mix; +Cer: Standard reaction mix containing 0.01 mM cerulenin.

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Table 9.  
Effect of Cofactors on Elongation Products of EL2<sup>1</sup>

Prod.	+Glu <sup>2</sup>	+Gal <sup>2</sup>	-ATP <sup>3</sup>	-CoA <sup>3</sup>	-A&C <sup>3</sup>	+Cer <sup>3</sup>
20:0	54.5	47.1	34.1	45.3	38.0	41.8
22:0	17.1	19.1	16.4	19.2	15.9	16.1
24:0	5.8	19.4	20.8	19.9	18.4	20.4
26:0	22.6	14.5	28.9	15.8	27.8	21.8
Total	100.0	100.0	100.0	100.0	100.0	100.0

<sup>1</sup> Amount of indicated product as a percent of total products formed. Results of one experiment for +Glucose; Average of two experiments for other conditions.

<sup>2</sup> +Glu: microsomes from cultures grown in the presence of glucose and incubated in standard reaction mix; +Gal: microsomes from cultures grown in the presence of galactose and incubated in standard reaction mix.

<sup>3</sup> Microsomes from galactose-induced cultures. -ATP: ATP omitted from reaction mix; -CoA: Coenzyme A omitted from reaction mix; -A&C: ATP and Coenzyme A omitted from reaction mix; +Cer: Standard reaction mix containing 0.01 mM cerulenin.

The results in Table 7 indicate that omission of ATP and/or CoA from the incubation mixture does not have a significant effect on the overall amounts of VLCFAs synthesized by the *in vitro* KAS activity of EL1 or EL2. The results also show that cerulenin does not inhibit the KAS activity of EL1 or EL2. The data in Table 8 and 9 confirm that EL1 and EL2 KAS activity produces significant amounts of C24:0 and C26:0 acyl CoA products.

To the extent not already indicated, it will be understood by those of ordinary skill in the art that any one of the various specific embodiments herein described and illustrated may be further modified to incorporate features shown in other of the specific embodiments.

The foregoing detailed description has been provided for a better understanding of the invention only and no unnecessary limitation should be understood therefrom as some modifications will be apparent to those

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skilled in the art without deviating from the spirit and scope of the appended claims.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION

- (i) APPLICANT: CARGILL, INCORPORATED
- (ii) TITLE OF THE INVENTION: FATTY ACID ELONGASES
- (iii) NUMBER OF SEQUENCES: 14
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Fish & Richardson P.C., P.A.
  - (B) STREET: 60 South Sixth Street, Suite 3300
  - (C) CITY: Minneapolis
  - (D) STATE: MN
  - (E) COUNTRY: USA
  - (F) ZIP: 55402
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Diskette
  - (B) COMPUTER: IBM Compatible
  - (C) OPERATING SYSTEM: DOS
  - (D) SOFTWARE: FastSEQ for Windows Version 2.0
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: 08/868,373
  - (B) FILING DATE: 03-JUN-1997
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Lundquist, Ronald C
  - (B) REGISTRATION NUMBER: 37,875
  - (C) REFERENCE/DOCKET NUMBER: 07039/064WO1
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 612-335-5050
  - (B) TELEFAX: 612-288-9696
  - (C) TELEX:

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1560 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: Genomic DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGGATCGAG	AGAGATTAAC	GGCGGAGATG	GCGTTTCGAG	ATTCATCATC	GGCCGTTATA	60
AGAATTCGAA	GACGTTTGCC	GGATTTATTA	ACGTCCGTTA	AGCTCAAATA	CGTGAAGCTT	120
GGACTTCACA	ACTCTTGCAA	CGTGACCACC	ATTCTCTTCT	TCTTAATTAT	TCTTCCTTTA	180
ACCGGAACCG	TGCTGGTTCA	GCTAACCGGT	CTAACGTTTCG	ATACGTTCTC	TGAGCTTTGG	240
TCTAACCAGG	CGGTTCAACT	CGACACGGCG	ACGAGACTTA	CCTGCTTGGT	TTTCCTCTCC	300
TTCGTTTTGA	CCCTCTACGT	GGCTAACCGG	TCTAAACCGG	TTACCTAGT	GGATTCTCC	360
TGCTACAAAC	CGGAAGACGA	GCGTAAAATA	TCAGTAGATT	CGTTCCTTGAC	GATGACTGAG	420
GAAAATGGAT	CATTCACCGA	TGACACGGTT	CAGTTCCAGC	AAAGAATCTC	GAACCGGGCC	480

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GGTTTGGGAG	ACGAGACGTA	TCTGCCACGT	GGCATAACTT	CAACGCCCCC	GAAGCTAAAT	540
ATGTCAGAGG	CACGTGCCGA	AGCTGAAGCC	GTTATGTTTG	GAGCCTTAGA	TTCCCTCTTC	600
GAGAAAACCG	GAATTAAACC	GGCCGAAGTC	GGAATCTTGA	TAGTAAACTG	CAGCTTATTC	660
AATCCGACGC	CGTCTCTATC	AGCGATGATC	GTGAACCATT	ACAAGATGAG	AGAAGACATC	720
AAAAGTTACA	ACCTCGGAGG	AATGGGTTGC	TCCGCCGGAT	TAATCTCAAT	CGATCTCGCT	780
AACAATCTCC	TCAAAGCAAA	CCCTAATTCT	TACGCTGTCG	TGGTAAGCAC	GGAAAACATA	840
ACCCTAAACT	GGTACTTCGG	AAATGACCGG	TCAATGCTCC	TCTGCAACTG	CATCTTCCGA	900
ATGGGCGGAG	CTGCGATTCT	CCTCTCTAAC	CGCCGTCAAG	ACCGGAAGAA	GTCAAAGTAC	960
TCGTGGTCA	ACGTCGTTTC	AACACATAAA	GGATCAGACG	ACAAGAACTA	CAATTGCGTG	1020
TACCAGAAGG	AAGACGAGAG	AGGAACAATC	GGTGTCTCTT	TAGCTAGAGA	GCTCATGTCT	1080
GTCCGCCGAG	ACGCTCTGAA	AACAAACATC	ACGACTTAG	GACCGATGGT	TCTTCCATTG	1140
TCAGAGCAGT	TGATGTTCTT	GATTTCCTTG	GTCAAAAGGA	AGATGTTCAA	GTTAAAAGTT	1200
AAACCGTATA	TTCCGGATTT	CAAGCTAGCT	TTCGAGCATT	TCTGTATTCA	CGCAGGAGGT	1260
AGAGCGGTTT	TAGACGAAAT	GCAGAAGAAT	CTTGATCTCA	AAGATTGGCA	CATGGAACCT	1320
TCTAGAATGA	CTTTGCACAG	ATTTGGTAAC	ACTTCGAGTA	GCTCGCTTTG	GTATGAGATG	1380
GCTTATACCG	AAGCTAAGGG	TCGGGTTAAA	GCTGGTGACC	GACTTTGGCA	GATTGCGTTT	1440
GGATCGGGTT	TCAAGTGTA	TAGTGCAGTT	TGGAAGCGT	TACGACCGGT	TTCCAGCGGAG	1500
GAGATGACCG	GTAATGCTTG	GGCTGGTTTC	ATTGATCAAT	ATCCGGTTAA	AGTTGTGCAA	1560

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 520 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Asp	Arg	Glu	Arg	Leu	Thr	Ala	Glu	Met	Ala	Phe	Arg	Asp	Ser	Ser	1	5	10	15
Ser	Ala	Val	Ile	Arg	Ile	Arg	Arg	Arg	Leu	Pro	Asp	Leu	Leu	Thr	Ser	20	25	30	35
Val	Lys	Leu	Lys	Tyr	Val	Lys	Leu	Gly	Leu	His	Asn	Ser	Cys	Asn	Val	40	45	50	55
Thr	Thr	Ile	Leu	Phe	Phe	Leu	Ile	Ile	Leu	Pro	Leu	Thr	Gly	Thr	Val	60	65	70	75
Leu	Val	Gln	Leu	Thr	Gly	Leu	Thr	Phe	Asp	Thr	Phe	Ser	Glu	Leu	Trp	80	85	90	95
Ser	Asn	Gln	Ala	Val	Gln	Leu	Asp	Thr	Ala	Thr	Arg	Leu	Thr	Cys	Leu	100	105	110	115
Val	Phe	Leu	Ser	Phe	Val	Leu	Thr	Leu	Tyr	Val	Ala	Asn	Arg	Ser	Lys	120	125	130	135
Pro	Val	Tyr	Leu	Val	Asp	Phe	Ser	Cys	Tyr	Lys	Pro	Glu	Asp	Glu	Arg	140	145	150	155
Lys	Ile	Ser	Val	Asp	Ser	Phe	Leu	Thr	Met	Thr	Glu	Glu	Asn	Gly	Ser	160	165	170	175
Phe	Thr	Asp	Asp	Thr	Val	Gln	Phe	Gln	Gln	Arg	Ile	Ser	Asn	Arg	Ala	180	185	190	195
Gly	Leu	Gly	Asp	Glu	Thr	Tyr	Leu	Pro	Arg	Gly	Ile	Thr	Ser	Thr	Pro	200	205	210	215
Pro	Lys	Leu	Asn	Met	Ser	Glu	Ala	Arg	Ala	Glu	Ala	Glu	Ala	Val	Met	220	225	230	235
Phe	Gly	Ala	Leu	Asp	Ser	Leu	Phe	Glu	Lys	Thr	Gly	Ile	Lys	Pro	Ala	240	245	250	255
Glu	Val	Gly	Ile	Leu	Ile	Val	Asn	Cys	Ser	Leu	Phe	Asn	Pro	Thr	Pro	260	265	270	275
Ser	Leu	Ser	Ala	Met	Ile	Val	Asn	His	Tyr	Lys	Met	Arg	Glu	Asp	Ile	280	285	290	295
Lys	Ser	Tyr	Asn	Leu	Gly	Gly	Met	Gly	Cys	Ser	Ala	Gly	Leu	Ile	Ser	300	305	310	315
Ile	Asp	Leu	Ala	Asn	Asn	Leu	Leu	Lys	Ala	Asn	Pro	Asn	Ser	Tyr	Ala	320	325	330	335
Val	Val	Val	Ser	Thr	Glu	Asn	Ile	Thr	Leu	Asn	Trp	Tyr	Phe	Gly	Asn	340	345	350	355



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Asp Arg Ser Met Leu Leu Cys Asn Cys Ile Phe Arg Met Gly Gly Ala  
 290 295 300  
 Ala Ile Leu Leu Ser Asn Arg Arg Gln Asp Arg Lys Lys Ser Lys Tyr  
 305 310 315  
 Ser Leu Val Asn Val Arg Thr His Lys Gly Ser Asp Asp Lys Asn  
 325 330 335  
 Tyr Asn Cys Val Tyr Gln Lys Glu Asp Glu Arg Gly Thr Ile Gly Val  
 340 345 350  
 Ser Leu Ala Arg Glu Leu Met Ser Val Ala Gly Asp Ala Leu Lys Thr  
 355 360 365  
 Asn Ile Thr Thr Leu Gly Pro Met Val Leu Pro Leu Ser Glu Gln Leu  
 370 375 380  
 Met Phe Leu Ile Ser Leu Val Lys Arg Lys Met Phe Lys Leu Lys Val  
 385 390 395  
 Lys Pro Tyr Ile Pro Asp Phe Lys Leu Ala Phe Glu His Phe Cys Ile  
 405 410 415  
 His Ala Gly Gly Arg Ala Val Leu Asp Glu Val Gln Lys Asn Leu Asp  
 420 425 430  
 Leu Lys Asp Trp His Met Glu Pro Ser Arg Met Thr Leu His Arg Phe  
 435 440 445  
 Gly Asn Thr Ser Ser Ser Ser Leu Trp Tyr Glu Met Ala Tyr Thr Glu  
 450 455 460  
 Ala Lys Gly Arg Val Lys Ala Gly Asp Arg Leu Trp Gln Ile Ala Phe  
 465 470 475  
 Gly Ser Gly Phe Lys Cys Asn Ser Ala Val Trp Lys Ala Leu Arg Pro  
 485 490 495  
 Val Ser Thr Glu Glu Met Thr Gly Asn Ala Trp Ala Gly Ser Ile Asp  
 500 505 510  
 Gln Tyr Pro Val Lys Val Val Gln  
 515 520

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1479 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: Genomic DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGGATTACC	CCATGAAGAA	GGTAAAAATC	TTTTTCAACT	ACCTCATGGC	GCATCGCTTC	60
AAGCTCTGCT	TCTTACCATT	AATGGTTGCT	ATAGCCGTGG	AGGCGTCTCG	TCTTTCCACA	120
CAAGATCTCC	AAAACITTTA	CCTCTACTTA	CAAAACAACC	ACACATCTCT	AACCATGTTT	180
TTCTTTTACC	TCGCTCTCGG	GTCGACTCTT	TACCTCATGA	CCCGGCCCAA	ACCCGTTTAT	240
CTCGTTGACT	TTAGCTGCTA	CCTCCCACCG	TCGCATCTCA	AAGCCAGCAC	CCAGAGGATC	300
ATGCAACACG	TAAGGCTTGT	ACGAGAAGCA	GGCGCGTGGA	AGCAAGAGTC	CGATTACTTG	360
ATGGACTTCT	GCGAGAAGAT	TCTAGAACGT	TCCGGTCTAG	GCCAAGAGAC	GTACGTACCC	420
GAAGGTCTTC	AAACTTTGCC	ACTACAACAG	AATTTGGCTG	TATCACGTAT	AGAGACGGAG	480
GAAGTTATTA	TTGGTGCGGT	CGATAATCTG	TTTCGCAACA	CGGAATAAAG	CCCTAGTGAT	540
ATAGGTATAT	TGGTGGTGAA	TTCAAGCACT	TTTAATCCAA	CACCTTCGCT	ATCAAGTATC	600
TTAGTGAATA	AGTTTAAACT	TAGGGATAAT	ATAAAGAGCT	TGAATCTTGG	TGGGATGGGG	660
TGTAGCGCTG	GAGTCATCGC	TATCGATGCG	GCTAAGAGCT	TGTTACAAGT	TCATAGAAAC	720
ACTTATGCTC	TTGTGGTGAG	CACGGAGAAC	ATCACTCAAA	ACTTGTACAT	GGGTAACAAC	780
AAATCAATGT	TGGTTACAAA	CTGTTTGTTC	CGTATAGGTG	GGGCCGCGAT	TTTGCTTTCT	840
AACCGGTCTA	TAGATCGTAA	ACGCGCAAAA	TACGAGCTTG	TTCACACCGT	GCGGGTCCAT	900
ACCGGAGCAG	ATGACCGATC	CTATGAATGT	GCAACTCAAG	AAGAGGATGA	AGATGGCATA	960
GTTGGGGTTT	CCTTGTCAAA	GAATCTACCA	ATGGTAGCTG	CAAGAACCCT	AAAGATCAAT	1020
ATCGCAACTT	TGGTCCGCT	TGTTCTTCCC	ATAAGCGAGA	AGTTTCACTT	CTTTGTGAGG	1080
TTCTGTTAAA	AGAAGTTTCT	CAACCCCAAG	CTAAAGCATT	ACATTCCGGA	TTTCAAGCTC	1140
GCATTGAGC	ATTTCTGTAT	CCATGCGGGT	GGTAGAGCGC	TAATTGATGA	GATGGAGAAG	1200
AATCTTCATC	TAATCCACT	AGACGTTGAG	GCTTCAAGAA	TGACATTACA	CAGGTTTGGT	1260
AATACCTCTT	CGAGCTCCAT	TTGGTACGAG	TTGGCTTACA	CAGAAGCCAA	AGGAAGGATG	1320
ACGAAAGGAG	ATAGGATTTG	GCAGATTGCG	TTGGGGTCAG	GTTTTAAGTG	TAATAGTTCA	1380

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GTTTGGGTGG CTCTTCGTAA CGTCAAGCCT TCTACTAATA ATCCTTGGGA ACAGTGTCTA 1440  
 CACAAATATC CAGTTGAGAT CGATATAGAT TTAAAAGAG 1479

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 493 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Asp	Tyr	Pro	Met	Lys	Lys	Val	Lys	Ile	Phe	Phe	Asn	Tyr	Leu	Met
1				5					10					15	
Ala	His	Arg	Phe	Lys	Leu	Cys	Phe	Leu	Pro	Leu	Met	Val	Ala	Ile	Ala
			20					25					30		
Val	Glu	Ala	Ser	Arg	Leu	Ser	Thr	Gln	Asp	Leu	Gln	Asn	Phe	Tyr	Leu
		35					40					45			
Tyr	Leu	Gln	Asn	Asn	His	Thr	Ser	Leu	Thr	Met	Phe	Phe	Leu	Tyr	Leu
	50				55						60				
Ala	Leu	Gly	Ser	Thr	Leu	Tyr	Leu	Met	Thr	Arg	Pro	Lys	Pro	Val	Tyr
65					70					75					80
Leu	Val	Asp	Phe	Ser	Cys	Tyr	Leu	Pro	Pro	Ser	His	Leu	Lys	Ala	Ser
			85						90					95	
Thr	Gln	Arg	Ile	Met	Gln	His	Val	Arg	Leu	Val	Arg	Glu	Ala	Gly	Ala
			100					105						110	
Trp	Lys	Gln	Glu	Ser	Asp	Tyr	Leu	Met	Asp	Phe	Cys	Glu	Lys	Ile	Leu
		115					120					125			
Glu	Arg	Ser	Gly	Leu	Gly	Gln	Glu	Thr	Tyr	Val	Pro	Glu	Gly	Leu	Gln
	130				135						140				
Thr	Leu	Pro	Leu	Gln	Gln	Asn	Leu	Ala	Val	Ser	Arg	Ile	Glu	Thr	Glu
145					150					155					160
Glu	Val	Ile	Ile	Gly	Ala	Val	Asp	Asn	Leu	Phe	Arg	Asn	Thr	Gly	Ile
			165					170						175	
Ser	Pro	Ser	Asp	Ile	Gly	Ile	Leu	Val	Val	Asn	Ser	Ser	Thr	Phe	Asn
			180					185					190		
Pro	Thr	Pro	Ser	Leu	Ser	Ser	Ile	Leu	Val	Asn	Lys	Phe	Lys	Leu	Arg
	195						200					205			
Asp	Asn	Ile	Lys	Ser	Leu	Asn	Leu	Gly	Gly	Met	Gly	Cys	Ser	Ala	Gly
	210					215					220				
Val	Ile	Ala	Ile	Asp	Ala	Ala	Lys	Ser	Leu	Leu	Gln	Val	His	Arg	Asn
	225				230					235				240	
Thr	Tyr	Ala	Leu	Val	Ser	Thr	Glu	Asn	Ile	Thr	Gln	Asn	Leu	Tyr	
			245					250					255		
Met	Gly	Asn	Asn	Lys	Ser	Met	Leu	Val	Thr	Asn	Cys	Leu	Phe	Arg	Ile
		260						265					270		
Gly	Gly	Ala	Ala	Ile	Leu	Leu	Ser	Asn	Arg	Ser	Ile	Asp	Arg	Lys	Arg
	275						280					285			
Ala	Lys	Tyr	Glu	Leu	Val	His	Thr	Val	Arg	Val	His	Thr	Gly	Ala	Asp
	290					295					300				
Asp	Arg	Ser	Tyr	Glu	Cys	Ala	Thr	Gln	Glu	Glu	Asp	Glu	Asp	Gly	Ile
305					310					315				320	
Val	Gly	Val	Ser	Leu	Ser	Lys	Asn	Leu	Pro	Met	Val	Ala	Ala	Arg	Thr
			325						330					335	
Leu	Lys	Ile	Asn	Ile	Ala	Thr	Leu	Gly	Pro	Leu	Val	Leu	Pro	Ile	Ser
		340						345					350		
Glu	Lys	Phe	His	Phe	Phe	Val	Arg	Phe	Val	Lys	Lys	Lys	Phe	Leu	Asn
	355					360						365			
Pro	Lys	Leu	Lys	His	Tyr	Ile	Pro	Asp	Phe	Lys	Leu	Ala	Phe	Glu	His
	370					375					380				
Phe	Cys	Ile	His	Ala	Gly	Arg	Ala	Leu	Ile	Asp	Glu	Met	Glu	Lys	
385					390					395				400	
Asn	Leu	His	Leu	Thr	Pro	Leu	Asp	Val	Glu	Ala	Ser	Arg	Met	Thr	Leu
			405						410					415	

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His Arg Phe Gly Asn Thr Ser Ser Ser Ser Ile Trp Tyr Glu Leu Ala  
 420 425 430  
 Tyr Thr Glu Ala Lys Gly Arg Met Thr Lys Gly Asp Arg Ile Trp Gln  
 435 440 445  
 Ile Ala Leu Gly Ser Gly Phe Lys Cys Asn Ser Ser Val Trp Val Ala  
 450 455 460  
 Leu Arg Asn Val Lys Pro Ser Thr Asn Asn Pro Trp Glu Gln Cys Leu  
 465 470 475 480  
 His Lys Tyr Pro Val Glu Ile Asp Ile Asp Leu Lys Glu  
 485 490

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1512 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: Genomic DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTACGTCAGG	GTAGAACAAA	GAGTAAACAC	TTAAGCAAAA	CAATTGTGTC	TACTCTTAGG	60
TTATCTCCAA	TGAAGAACTT	AAAGATGGTT	TTCTTCAAGA	TCCTCTTTAT	CTCTTTAATG	120
GCAGGATTAG	CCATGAAAGG	ATCTAAGATC	AACGTAGAAG	ATCTCCAAAA	GTTCTCCCTC	180
CACCATACAC	AGAACAACCT	CCAAACCATA	AGCCTTCTAT	TGTTTCTTGT	CGTTTTTGTG	240
TGGATCCTCT	ACATGTTAAC	CCGACCTAAA	CCCGTTTACC	TTGTTGATTT	CTCCTGCTAC	300
CTTCCACCGT	CGCATCTCAA	GGTCAGTATC	CAAACCCTAA	TGGGACACGC	AAGACGTGCA	360
AGAGAAGCAG	GCATGTGTG	GAAGAACAAA	GAGAGCGACC	ATTTAGTTGA	CTTCCAGGAG	420
AAGATTCTTG	AACGTTCCGG	TCTTGGTCAA	GAAACCTACA	TCCCCGAGGG	TCTTCAGTGC	480
TTCCCACTTC	AGCAAGGCAT	GGGTGCTTCA	CGTAAAGAGA	CGGAAGAAGT	AATCTTCGGA	540
GCTCTTGACA	ATCTTTTTTCG	CAACACCGGT	GTA AACCTG	ATGATATCGG	TATATTGGTG	600
GTGAATTCTA	GCACGTTTAA	TCCAACCTCA	TCACTCGCCT	CCATGATTGT	GAACAAGTAC	660
AAACTCAGAG	ACAACATCAA	GAGTTTGAAT	CTTGGAGGGA	TGGGTTGCAG	TGCCGGAGTT	720
ATAGCTGTTG	ATGTCGCTAA	GGGATTACTA	CAAGTTCATA	GGAACACTTA	TGCTATTGTA	780
GTAAGCACAG	AGAACATCAC	TCAGAACTTA	TACTTGGGGA	AAAACAAATC	AATGCTAGTC	840
ACAAACTGTT	TGTTCCGCGT	TGGTGGTGCT	GCGGTTCTGC	TTTCAAACAG	ATCTAGAGAC	900
CGTAACCGCG	CCAAATACGA	GCTTGTTTAC	ACCGTACGGA	TCCATACCGG	ATCAGATGAT	960
AGTTCGTTTC	AATGTGCGAC	ACAAGAAGAG	GATGAAGATG	GTATAATTGG	AGTTACCTTG	1020
ACAAAGAATC	TACCTATGGT	GGCTGCAAGG	ACTCTTAAGA	TAAATATCGC	AACTTTGGGT	1080
CCTCTTGATC	TTCCATTAAA	AGAGAAGCTA	GCCTTCTTTA	TTACTTTTGT	CAAGAAGAAG	1140
TATTTCAAGC	CAGAGTTAAG	GAATTATACA	CCAGATTTCA	AGCTTGCCTT	TGAGCATTTT	1200
TGTATCCACG	CTGGTGGGAG	AGCTTAATA	GATGAGCTGG	AGAAGAACCT	TAAGCTTTCT	1260
CCGTTACACG	TAGAGGCGTC	AAGAATGACA	CTACACAGGT	TTGGTAACAC	TTCTTCTAGC	1320
TCAATCTGGT	ACGAGTTAGC	TTATACAGAA	GCTAAAGGAA	GGATGAAGGA	AGGAGATAGG	1380
ATTTGGCAGA	TTGCTTTGGG	GTCAGGTTTT	AAGTGTAAAC	GTTCAAGTATG	GGTGGCTCTG	1440
CGAGACGTTA	AGCCTTCAGC	TAACAGTCCA	TGGGAAGACT	GTATGGATAG	ATATCCGGTT	1500
GAGATTGATA	TT					1512

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 504 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Leu Arg Gln Gly Arg Thr Lys Ser Lys His Leu Ser Lys Thr Ile Cys  
 1 5 10 15  
 Pro Thr Leu Arg Leu Ser Pro Met Lys Asn Leu Lys Met Val Phe Phe  
 20 25 30

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Lys Ile Leu Phe Ile Ser Leu Met Ala Gly Leu Ala Met Lys Gly Ser  
 35 40 45  
 Lys Ile Asn Val Glu Asp Leu Gln Lys Phe Ser Leu His His Thr Gln  
 50 55 60  
 Asn Asn Leu Gln Thr Ile Ser Leu Leu Leu Phe Leu Val Val Phe Val  
 65 70 75 80  
 Trp Ile Leu Tyr Met Leu Thr Arg Pro Lys Pro Val Tyr Leu Val Asp  
 85 90 95  
 Phe Ser Cys Tyr Leu Pro Pro Ser His Leu Lys Val Ser Ile Gln Thr  
 100 105 110  
 Leu Met Gly His Ala Arg Arg Ala Arg Glu Ala Gly Met Cys Trp Lys  
 115 120 125  
 Asn Lys Glu Ser Asp His Leu Val Asp Phe Gln Glu Lys Ile Leu Glu  
 130 135 140  
 Arg Ser Gly Leu Gly Gln Glu Thr Tyr Ile Pro Glu Gly Leu Gln Cys  
 145 150 155 160  
 Phe Pro Leu Gln Gln Gly Met Gly Ala Ser Arg Lys Glu Thr Glu Glu  
 165 170 175  
 Val Ile Phe Gly Ala Leu Asp Asn Leu Phe Arg Asn Thr Gly Val Lys  
 180 185 190  
 Pro Asp Asp Ile Gly Ile Leu Val Asn Ser Ser Thr Phe Asn Pro  
 195 200 205  
 Thr Pro Ser Leu Ala Ser Met Ile Val Asn Lys Tyr Lys Leu Arg Asp  
 210 215 220  
 Asn Ile Lys Ser Leu Asn Leu Gly Gly Met Gly Cys Ser Ala Gly Val  
 225 230 235 240  
 Ile Ala Val Asp Val Ala Lys Gly Leu Leu Gln Val His Arg Asn Thr  
 245 250 255  
 Tyr Ala Ile Val Val Ser Thr Glu Asn Ile Thr Gln Asn Leu Tyr Leu  
 260 265 270  
 Gly Lys Asn Lys Ser Met Leu Val Thr Asn Cys Leu Phe Arg Val Gly  
 275 280 285  
 Gly Ala Ala Val Leu Leu Ser Asn Arg Ser Arg Asp Arg Asn Arg Ala  
 290 295 300  
 Lys Tyr Glu Leu Val His Thr Val Arg Ile His Thr Gly Ser Asp Asp  
 305 310 315 320  
 Arg Ser Phe Glu Cys Ala Thr Gln Glu Glu Asp Glu Asp Gly Ile Ile  
 325 330 335  
 Gly Val Thr Leu Thr Lys Asn Leu Pro Met Val Ala Ala Arg Thr Leu  
 340 345 350  
 Lys Ile Asn Ile Ala Thr Leu Gly Pro Leu Val Leu Pro Leu Lys Glu  
 355 360 365  
 Lys Leu Ala Phe Phe Ile Thr Phe Val Lys Lys Lys Tyr Phe Lys Pro  
 370 375 380  
 Glu Leu Arg Asn Tyr Thr Pro Asp Phe Lys Leu Ala Phe Glu His Phe  
 385 390 395 400  
 Cys Ile His Ala Gly Gly Arg Ala Leu Ile Asp Glu Leu Glu Lys Asn  
 405 410 415  
 Leu Lys Leu Ser Pro Leu His Val Glu Ala Ser Arg Met Thr Leu His  
 420 425 430  
 Arg Phe Gly Asn Thr Ser Ser Ser Ile Trp Tyr Glu Leu Ala Tyr  
 435 440 445  
 Thr Glu Ala Lys Gly Arg Met Lys Glu Gly Asp Arg Ile Trp Gln Ile  
 450 455 460  
 Ala Leu Gly Ser Gly Phe Lys Cys Asn Ser Ser Val Trp Val Ala Leu  
 465 470 475 480  
 Arg Asp Val Lys Pro Ser Ala Asn Ser Pro Trp Glu Asp Cys Met Asp  
 485 490 495  
 Arg Tyr Pro Val Glu Ile Asp Ile  
 500

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## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1650 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: Genomic DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

ATGGGTAGAT CCAACGAGCA AGATCTGCTC TCTACCGAGA TCGTTAATCG TGGGATCGAA      60
CCATCCGGTC CTAACGCCGG CTCACCAACG TTCTCGGTGA GGGTCAGGAG ACGTTTGCCT      120
GATTTTCTTC AGTCGGTGAA CTTGAAGTAC GTGAAACTTG GTTACCACTA CCTCATAAAC      180
CATGCGGTTT ATTTGGCGAC CATACCGGTT CTTGTGCTGG TTTTGTAGTC TGAGGTTGGG      240
AGTTTAAAGCA GAGAAGAGAT TTGGAAGAAG CTTTGGGACT ATGATCTTGC AACTGTTATC      300
GGATTCTTCG GTGTCTTTGT TTTAACCGCT TGTGTCTACT TCATGTCTCG TCCTCGCTCT      360
GTTTATCTTA TTGATTTTCG TTGTTACAAG CCCTCCGATG AACACAAGGT GACAAAAGAA      420
GAGTTCATAG AACTAGCGAG AAAATCAGGG AAGTTCGACG AAGAGACACT CGGTTTCAAG      480
AAGAGGATCT TACAAGCCTC AGGCATAGGC GACGAGACAT ACGTCCCAAG ATCCATCTCT      540
TCATCAGAAA ACATAACAAC GATGAAAGAA GGTCTGTGAAG AAGCCTCTAC AGTGATCTTT      600
GGAGCACTAG ACGAACTCTT CGAGAAGACA CGTGTAAGAA CTAAAGACGT TGGTGTCTCT      660
GTGGTTAACT GTAGCATTTT CAACCCGACA CCGTCGTTGT CCGCAATGGT GATAAACCAT      720
TACAAGATGA GAGGGAACAT ACTTAGTTAC AACCTTGGAG GGATGGGATG TTCGGCTGGA      780
ATCATAGCTA TTGATCTTGC TCGTGACATG CTTCAGTCTA ACCCTAATAG TTATGCTGTT      840
GTTGTGAGTA CTGAGATGGT TGGGTATAAT TGGTACGTGG GAAGTGACAA GTCAATGGTT      900
ATACCTAATT GTTCTTTAG GATGGGTTGT TCTGCCGTTA TGCTCTCTAA CCGTCGTCGT      960
GACTTTCGCC ATGCTAAGTA CCGTCTCGAG CACATTGTCC GAACTCATAA GGCTGCTGAC      1020
GACCGTAGCT TCAGGAGTGT GTACCAGGAA GAAGATGAAC AAGGATTCAA GGGGTTGAAG      1080
ATAAGTAGAG ACTTAATGGA AGTTGGAGGT GAAGCTCTCA AGACAAACAT CACTACCTTA      1140
GGTCCTCTTG TCCTACCTTT CTCCGAGCAG CTTCTCTTCT TTGCTGCTTT GGTCCGCCGA      1200
ACATTCTCAC CTGCTGCCAA AACGTCCACA ACCACTTCCT TCTCTACTTC CGCCACCGCA      1260
AAAACCAATG GAATCAAGTC TTCCTCTTCC GATCTGTCCA AGCCATACAT CCCGACTAC      1320
AAGCTCGCCT TCGAGCATTT TTGCTTCCAC GCGGCAAGCA AAGTAGTGCT TGAAGAGCTT      1380
CAAAAGAATC TAGGCTTGAG TGAAGAGAAT ATGGAGGCTT CTAGGATGAC ACTTCACAGG      1440
TTTGGAACA CTTCTAGCAG TGGAATCTGG TATGAGTTGG CTTACATGGA GGCCAAGGAA      1500
AGTGTTCGTA GAGGCGATAG GGTTTGGCAG ATCGCTTTCG GTTCTGGTTT TAAGTGTAAAC      1560
AGTGTGGTGT GGAAGGCAAT GAGGAAGGTG AAGAAGCCAA CCAGGAACAA TCCTTGGGTG      1620
GATTGCATCA ACCGTTACCC TGTGCCTCTC

```

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 550 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: None

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```

Met Gly Arg Ser Asn Glu Gln Asp Leu Leu Ser Thr Glu Ile Val Asn
 1           5           10           15
Arg Gly Ile Glu Pro Ser Gly Pro Asn Ala Gly Ser Pro Thr Phe Ser
 20           25           30
Val Arg Val Arg Arg Arg Leu Pro Asp Phe Leu Gln Ser Val Asn Leu
 35           40           45
Lys Tyr Val Lys Leu Gly Tyr His Tyr Leu Ile Asn His Ala Val Tyr
 50           55           60
Leu Ala Thr Ile Pro Val Leu Val Leu Val Phe Ser Ala Glu Val Gly
 65           70           75           80
Ser Leu Ser Arg Glu Glu Ile Trp Lys Lys Leu Trp Asp Tyr Asp Leu
 85           90           95
Ala Thr Val Ile Gly Phe Phe Gly Val Phe Val Leu Thr Ala Cys Val
100           105           110

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Tyr Phe Met Ser Arg Pro Arg Ser Val Tyr Leu Ile Asp Phe Ala Cys  
 115 120 125  
 Tyr Lys Pro Ser Asp Glu His Lys Val Thr Lys Glu Glu Phe Ile Glu  
 130 135 140  
 Leu Ala Arg Lys Ser Gly Lys Phe Asp Glu Glu Thr Leu Gly Phe Lys  
 145 150 155 160  
 Lys Arg Ile Leu Gln Ala Ser Gly Ile Gly Asp Glu Thr Tyr Val Pro  
 165 170 175  
 Arg Ser Ile Ser Ser Ser Glu Asn Ile Thr Thr Met Lys Glu Gly Arg  
 180 185 190  
 Glu Glu Ala Ser Thr Val Ile Phe Gly Ala Leu Asp Glu Leu Phe Glu  
 195 200 205  
 Lys Thr Arg Val Lys Pro Lys Asp Val Gly Val Leu Val Val Asn Cys  
 210 215 220  
 Ser Ile Phe Asn Pro Thr Pro Ser Leu Ser Ala Met Val Ile Asn His  
 225 230 235 240  
 Tyr Lys Met Arg Gly Asn Ile Leu Ser Tyr Asn Leu Gly Gly Met Gly  
 245 250 255  
 Cys Ser Ala Gly Ile Ile Ala Ile Asp Leu Ala Arg Asp Met Leu Gln  
 260 265 270  
 Ser Asn Pro Asn Ser Tyr Ala Val Val Ser Thr Glu Met Val Gly  
 275 280 285  
 Tyr Asn Trp Tyr Val Gly Ser Asp Lys Ser Met Val Ile Pro Asn Cys  
 290 295 300  
 Phe Phe Arg Met Gly Cys Ser Ala Val Met Leu Ser Asn Arg Arg Arg  
 305 310 315 320  
 Asp Phe Arg His Ala Lys Tyr Arg Leu Glu His Ile Val Arg Thr His  
 325 330 335  
 Lys Ala Ala Asp Asp Arg Ser Phe Arg Ser Val Tyr Gln Glu Glu Asp  
 340 345 350  
 Glu Gln Gly Phe Lys Gly Leu Lys Ile Ser Arg Asp Leu Met Glu Val  
 355 360 365  
 Gly Gly Glu Ala Leu Lys Thr Asn Ile Thr Thr Leu Gly Pro Leu Val  
 370 375 380  
 Leu Pro Phe Ser Glu Gln Leu Leu Phe Phe Ala Leu Val Arg Arg  
 385 390 395 400  
 Thr Phe Ser Pro Ala Ala Lys Thr Ser Thr Thr Thr Ser Phe Ser Thr  
 405 410 415  
 Ser Ala Thr Ala Lys Thr Asn Gly Ile Lys Ser Ser Ser Ser Asp Leu  
 420 425 430  
 Ser Lys Pro Tyr Ile Pro Asp Tyr Lys Leu Ala Phe Glu His Phe Cys  
 435 440 445  
 Phe His Ala Ala Ser Lys Val Val Leu Glu Glu Leu Gln Lys Asn Leu  
 450 455 460  
 Gly Leu Ser Glu Glu Asn Met Glu Ala Ser Arg Met Thr Leu His Arg  
 465 470 475 480  
 Phe Gly Asn Thr Ser Ser Ser Gly Ile Trp Tyr Glu Leu Ala Tyr Met  
 485 490 495  
 Glu Ala Lys Glu Ser Val Arg Arg Gly Asp Arg Val Trp Gln Ile Ala  
 500 505 510  
 Phe Gly Ser Gly Phe Lys Cys Asn Ser Val Val Trp Lys Ala Met Arg  
 515 520 525  
 Lys Val Lys Lys Pro Thr Arg Asn Asn Pro Trp Val Asp Cys Ile Asn  
 530 535 540  
 Arg Tyr Pro Val Pro Leu  
 545 550

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1611 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: Genomic DNA

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TCGAGCTACG	TCAGGGCTTT	TATATGCACA	AATTCTCATA	AAGTTTTCAA	TTTTATTCCA	60
TTTTTCTCGG	AAGCCATGGA	AGCTGCTAAT	GAGCCTGTTA	ATGGCGGATC	CGTACAGATC	120
CGAACAGAGA	ACAACGAAAG	ACGAAAGCTT	CCTAATTCTT	TACAAAGCGT	CAACATGAAA	180
TACGTCAAGC	TAGGTTATCA	TTACCTCATT	ACTCATCTCT	TCAAGCTCTG	TTTGGTTCCA	240
TTAATGGCGG	TTTGTAGTAC	AGAGATCTCT	CGATTAACAA	CAGACGATCT	TTACCAGATT	300
TGGCTTCATC	TCCAATACAA	TCTCGTTGCT	TTCATCTTTC	TCTCTGCTTT	AGCTATCTTT	360
GGCTCCACCG	TTTACATCAT	GAGTCGTCCC	AGATCTGTTT	ATCTCGTTGA	TTACTCTTGT	420
TATCTTCCTC	CGGAGAGTCT	TCAGGTTAAG	TATCAGAAGT	TTATGGATCA	TTCTAAGTTG	480
ATTGAAGATT	TCAATGAGTC	ATCTTTAGAG	TTTCAGAGGA	AGATTCTTGA	ACGTTCTGGT	540
TTAGGAGAAG	AGACTTATCT	CCCTGAAGCT	TTACATTGTA	TCCCTCCGAG	GCCTACGATG	600
ATGGCGGCTC	GTGAGGAATC	TGAGCAGGTA	ATGTTTGGTG	CTCTTGATAA	GCTTTTCGAG	660
AATACCAAGA	TTAACCCTAG	GGATATTGGT	GTGTTGGTTG	TGAATTGTAG	CTTGTTTAAT	720
CCTACACCTT	CGTTGTGAGC	TATGATTGTT	AACAAGTATA	AGCTTAGAGG	GAATGTTAAG	780
AGTTTTAACC	TTGGTGAAT	GGGGTGTAGT	GCTGGTGTTA	TCTCTATCGA	TTTAGCTAAA	840
GATATGTTGC	AAGTTCATAG	GAATACTTAT	GCTGTTGTGG	TTAGTACTGA	GAACATTACT	900
CAGAATTGGT	ATTTTGGGAA	TAAGAAGGCT	ATGTTGATTC	CGAATTGTTT	GTTTCGTGTT	960
GGTGGTTCGG	CGATTTTGTT	GTGGAACAAG	GGGAAAGATC	GTAGACGGTC	TAAGTATAAG	1020
CTTGTTTCATA	CCGTTAGGAC	TCATAAAGGA	GCTGTTGAGA	AGGCTTTCAA	CTGTGTTTAC	1080
CAAGAGCAAG	ATGATAATGG	GAAGACCGGG	GTTTCGTTGT	CGAAAGATCT	TATGGCTATA	1140
GCTGGGGAAG	CTCTTAAGGC	GAATATCACT	ACTTTAGGTC	CTTTGGTTCT	TCCTATAAGT	1200
GAGCAGATTC	TGTTTTTCAT	GACTTTGGTT	ACGAAGAAAC	TGTTTAACTC	GAAGCTGAAG	1260
CCGTATATTG	CGGATTTCAA	GCTTGCCTTT	GATCATTTCT	GTATCCATGC	TGGTGGTAGA	1320
GCTGTGATTG	ATGAGCTTGA	GAAGAATCTG	CAGCTTTCGC	AGACTCATGT	CGAGGCATCC	1380
AGAATGACAC	TGCACAGATT	TGGAAACACT	TCTTCGAGCT	CGATTTGGTA	TGAACTGGCT	1440
TACATAGAGG	CTAAAGGTAG	GATGAAGAAA	GGAAACCGGG	TTTGGCAGAT	TGCTTTTGGA	1500
AGTGGGTTTA	AGTGTAACAG	TGCAGTTTGG	GTGGCTCTAA	ACAATGTCAA	GCCTTCGGTT	1560
AGTAGTCCGT	GGGAACACTG	CATCGACCGA	TATCCGGTTA	AGCTCGACTT	C	1611

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 537 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ser	Ser	Tyr	Val	Arg	Ala	Phe	Ile	Cys	Thr	Asn	Ser	His	Lys	Val	Phe
1				5					10					15	
Asn	Phe	Ile	Pro	Phe	Phe	Ser	Glu	Ala	Met	Glu	Ala	Ala	Asn	Glu	Pro
			20				25						30		
Val	Asn	Gly	Gly	Ser	Val	Gln	Ile	Arg	Thr	Glu	Asn	Asn	Glu	Arg	Arg
		35				40						45			
Lys	Leu	Pro	Asn	Phe	Leu	Gln	Ser	Val	Asn	Met	Lys	Tyr	Val	Lys	Leu
	50				55					60					
Gly	Tyr	His	Tyr	Leu	Ile	Thr	His	Leu	Phe	Lys	Leu	Cys	Leu	Val	Pro
65				70					75					80	
Leu	Met	Ala	Val	Leu	Val	Thr	Glu	Ile	Ser	Arg	Leu	Thr	Thr	Asp	Asp
			85					90						95	
Leu	Tyr	Gln	Ile	Trp	Leu	His	Leu	Gln	Tyr	Asn	Leu	Val	Ala	Phe	Ile
		100				105						110			
Phe	Leu	Ser	Ala	Leu	Ala	Ile	Phe	Gly	Ser	Thr	Val	Tyr	Ile	Met	Ser
	115				120						125				
Arg	Pro	Arg	Ser	Val	Tyr	Leu	Val	Asp	Tyr	Ser	Cys	Tyr	Leu	Pro	Pro
	130				135					140					
Glu	Ser	Leu	Gln	Val	Lys	Tyr	Gln	Lys	Phe	Met	Asp	His	Ser	Lys	Leu
145				150					155					160	
Ile	Glu	Asp	Phe	Asn	Glu	Ser	Ser	Leu	Glu	Phe	Gln	Arg	Lys	Ile	Leu
		165				170							175		
Glu	Arg	Ser	Gly	Leu	Gly	Glu	Glu	Thr	Tyr	Leu	Pro	Glu	Ala	Leu	His
		180				185							190		

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Cys Ile Pro Pro Arg Pro Thr Met Met Ala Ala Arg Glu Glu Ser Glu  
 195 200 205  
 Gln Val Met Phe Gly Ala Leu Asp Lys Leu Phe Glu Asn Thr Lys Ile  
 210 215 220  
 Asn Pro Arg Asp Ile Gly Val Leu Val Val Asn Cys Ser Leu Phe Asn  
 225 230 235 240  
 Pro Thr Pro Ser Leu Ser Ala Met Ile Val Asn Lys Tyr Lys Leu Arg  
 245 250 255  
 Gly Asn Val Lys Ser Phe Asn Leu Gly Gly Met Gly Cys Ser Ala Gly  
 260 265 270  
 Val Ile Ser Ile Asp Leu Ala Lys Asp Met Leu Gln Val His Arg Asn  
 275 280 285  
 Thr Tyr Ala Val Val Val Ser Thr Glu Asn Ile Thr Gln Asn Trp Tyr  
 290 295 300  
 Phe Gly Asn Lys Lys Ala Met Leu Ile Pro Asn Cys Leu Phe Arg Val  
 305 310 315 320  
 Gly Gly Ser Ala Ile Leu Leu Ser Asn Lys Gly Lys Asp Arg Arg Arg  
 325 330 335  
 Ser Lys Tyr Lys Leu Val His Thr Val Arg Thr His Lys Gly Ala Val  
 340 345 350  
 Glu Lys Ala Phe Asn Cys Val Tyr Gln Glu Gln Asp Asp Asn Gly Lys  
 355 360 365  
 Thr Gly Val Ser Leu Ser Lys Asp Leu Met Ala Ile Ala Gly Glu Ala  
 370 375 380  
 Leu Lys Ala Asn Ile Thr Thr Leu Gly Pro Leu Val Leu Pro Ile Ser  
 385 390 395 400  
 Glu Gln Ile Leu Phe Phe Met Thr Leu Val Thr Lys Lys Leu Phe Asn  
 405 410 415  
 Ser Lys Leu Lys Pro Tyr Ile Pro Asp Phe Lys Leu Ala Phe Asp His  
 420 425 430  
 Phe Cys Ile His Ala Gly Gly Arg Ala Val Ile Asp Glu Leu Glu Lys  
 435 440 445  
 Asn Leu Gln Leu Ser Gln Thr His Val Glu Ala Ser Arg Met Thr Leu  
 450 455 460  
 His Arg Phe Gly Asn Thr Ser Ser Ser Ser Ile Trp Tyr Glu Leu Ala  
 465 470 475 480  
 Tyr Ile Glu Ala Lys Gly Arg Met Lys Lys Gly Asn Arg Val Trp Gln  
 485 490 495  
 Ile Ala Phe Gly Ser Gly Phe Lys Cys Asn Ser Ala Val Trp Val Ala  
 500 505 510  
 Leu Asn Asn Val Lys Pro Ser Val Ser Ser Pro Trp Glu His Cys Ile  
 515 520 525  
 Asp Arg Tyr Pro Val Lys Leu Asp Phe  
 530 535

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1502 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: Genomic DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TCTCCGACGA	TGCCTCAGGC	ACCGATGCCA	GAGTTCTCTA	GCTCGGTGAA	GCTCAAGTAC	60
GTGAAACTTG	GTTACCAATA	TTGGTTAAC	CATTTCTTGA	GTTTTCTTTT	GATCCCGATC	120
ATGGCTATTG	TCGCCGTTGA	GCTTCTTCGG	ATGGGTCCTG	AAGAGATCCT	TAATGTTTGG	180
AATTCACCTC	AGTTTGACCT	AGTTCAGGTT	CTATGTTCTT	CCTTCTTTGT	CATCTTCATC	240
TCCACTGTTT	ACTTCATGTC	CAAGCCACGC	ACCATCTACC	TCGTTGACTA	TTCTTGTTAC	300
AAGCCACCTG	TCACGTGTCG	TGTCCCTTC	GCAACTTTCA	TGGAACACTC	TCGTTTGATC	360
CTCAAGGACA	AGCCTAAGAG	CGTCGAGTTC	CAAATGAGAA	TCCTTGAACG	TTCTGGCCTC	420
GGTGAGGAGA	CTTGCTCTCC	TCCGGCTATT	CATTATATTC	CTCCACACC	AACCATGGAC	480
GCGGCTAGAA	GCGAGGCTCA	GATGGTTATC	TTGAGGCCA	TGGACGATCT	TTTCAAGAAA	540
ACCGGTCTTA	AACCTAAAGA	CGTCGACATC	CTTATCGTCA	ACTGCTCTCT	TTTCTCTCCC	600



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ACACCATCGC	TCTCAGCTAT	GGTCATCAAC	AAATATAAGC	TTAGGAGTAA	TATCAAGAGC	660
TTCAATCTTT	CGGGGATGGG	CTGCAGCGCG	GGCCTGATCT	CAGTTGATCT	AGCCCGCGAC	720
TTGCTCCAAG	TTTCATCCCA	TTCAAATGCA	ATCATCGTCA	GCACGGAGAT	CATAACGCCT	780
AATTACTATC	AAGGCAACGA	GAGAGCCATG	TTGTTACCCA	ATTGTCTCTT	CCGCATGGGT	840
GCGGCAGCCA	TACACATGTC	AAACCGCCGG	TCTGACCGGT	GGCGAGCCAA	ATACAAGCTT	900
TCCACCTCG	TCCGGACACA	CCGTGGCGCT	GACGACAAGT	CTTTCTACTG	TGTCTACGAA	960
CAGGAAGACA	AAGAAGGACA	CGTTGGCATC	AACTTGTTCA	AAGATCTCAT	GGCCATCGCC	1020
GGTGAAGCCC	TCAAGGCAAA	CATCACCACA	ATAGGTCCTT	TGGTCCTACC	GGCGTCAGAA	1080
CAACTTCTCT	TCCTCACGTC	CCTAATCGGA	CGTAAATCT	TCAACCCGAA	ATGGAAACCA	1140
TACATACCGG	ATTTCAAGCT	GGCCTTCGAA	CACTTTGTCA	TTCACGCAGG	AGGCAGAGCG	1200
GTGATCGACG	AGCTCCAAAA	GAATCTACAA	CTATCAGGAG	AACACGTTGA	GGCCTCAAGA	1260
ATGACACTAC	ATCGTTTGG	TAACACGTCA	TCTTCATCGT	TATGGTACGA	GCTTAGCTAC	1320
ATCGAGTCTA	AAGGGAGAAT	GAGGAGAGGC	GATCGCGTTT	GGCAAATCGC	GTTTGGGAGT	1380
GGTTTCAAGT	GTAACCTCTG	CGTGTGGAAG	TGTAACCGTA	CGATTAAGAC	ACCTAAGGAC	1440
GGACCATGGT	CCGATTGTAT	CGACCGTTAC	CCTGTCTTTA	TTCCCGAAGT	TGTCAAACCTC	1500
TA						1502

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 500 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ser	Pro	Thr	Met	Pro	Gln	Ala	Pro	Met	Pro	Glu	Phe	Ser	Ser	Ser	Val
1				5					10					15	
Lys	Leu	Lys	Tyr	Val	Lys	Leu	Gly	Tyr	Gln	Tyr	Leu	Val	Asn	His	Phe
			20					25					30		
Leu	Ser	Phe	Leu	Leu	Ile	Pro	Ile	Met	Ala	Ile	Val	Ala	Val	Glu	Leu
			35					40				45			
Leu	Arg	Met	Gly	Pro	Glu	Glu	Ile	Leu	Asn	Val	Trp	Asn	Ser	Leu	Gln
			50			55					60				
Phe	Asp	Leu	Val	Gln	Val	Leu	Cys	Ser	Ser	Phe	Phe	Val	Ile	Phe	Ile
				70						75				80	
Ser	Thr	Val	Tyr	Phe	Met	Ser	Lys	Pro	Arg	Thr	Ile	Tyr	Leu	Val	Asp
				85					90					95	
Tyr	Ser	Cys	Tyr	Lys	Pro	Pro	Val	Thr	Cys	Arg	Val	Pro	Phe	Ala	Thr
				100				105					110		
Phe	Met	Glu	His	Ser	Arg	Leu	Ile	Leu	Lys	Asp	Lys	Pro	Lys	Ser	Val
				115				120				125			
Glu	Phe	Gln	Met	Arg	Ile	Leu	Glu	Arg	Ser	Gly	Leu	Gly	Glu	Glu	Thr
						135					140				
Cys	Leu	Pro	Pro	Ala	Ile	His	Tyr	Ile	Pro	Pro	Thr	Pro	Thr	Met	Asp
				150						155				160	
Ala	Ala	Arg	Ser	Glu	Ala	Gln	Met	Val	Ile	Phe	Glu	Ala	Met	Asp	Asp
				165					170					175	
Leu	Phe	Lys	Lys	Thr	Gly	Leu	Lys	Pro	Lys	Asp	Val	Asp	Ile	Leu	Ile
				180				185						190	
Val	Asn	Cys	Ser	Leu	Phe	Ser	Pro	Thr	Pro	Ser	Leu	Ser	Ala	Met	Val
				195				200				205			
Ile	Asn	Lys	Tyr	Lys	Leu	Arg	Ser	Asn	Ile	Lys	Ser	Phe	Asn	Leu	Ser
				210			215					220			
Gly	Met	Gly	Cys	Ser	Ala	Gly	Leu	Ile	Ser	Val	Asp	Leu	Ala	Arg	Asp
						230				235				240	
Leu	Leu	Gln	Val	His	Pro	Asn	Ser	Asn	Ala	Ile	Ile	Val	Ser	Thr	Glu
				245					250					255	
Ile	Ile	Thr	Pro	Asn	Tyr	Tyr	Gln	Gly	Asn	Glu	Arg	Ala	Met	Leu	Leu
				260				265					270		
Pro	Asn	Cys	Leu	Phe	Arg	Met	Gly	Ala	Ala	Ala	Ile	His	Met	Ser	Asn
				275				280				285			
Arg	Arg	Ser	Asp	Arg	Trp	Arg	Ala	Lys	Tyr	Lys	Leu	Ser	His	Leu	Val
				290			295				300				

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Arg Thr His Arg Gly Ala Asp Asp Lys Ser Phe Tyr Cys Val Tyr Glu  
 305 310 315 320  
 Gln Glu Asp Lys Gly His Val Gly Ile Asn Leu Ser Lys Asp Leu  
 325 330 335  
 Met Ala Ile Ala Gly Glu Ala Leu Lys Ala Asn Ile Thr Thr Ile Gly  
 340 345 350  
 Pro Leu Val Leu Pro Ala Ser Glu Gln Leu Leu Phe Leu Thr Ser Leu  
 355 360 365  
 Ile Gly Arg Lys Ile Phe Asn Pro Lys Trp Lys Pro Tyr Ile Pro Asp  
 370 375 380  
 Phe Lys Leu Ala Phe Glu His Phe Cys Ile His Ala Gly Gly Arg Ala  
 385 390 395 400  
 Val Ile Asp Glu Leu Gln Lys Asn Leu Gln Leu Ser Gly Glu His Val  
 405 410 415  
 Glu Ala Ser Arg Met Thr Leu His Arg Phe Gly Asn Thr Ser Ser Ser  
 420 425 430  
 Ser Leu Trp Tyr Glu Leu Ser Tyr Ile Glu Ser Lys Gly Arg Met Arg  
 435 440 445  
 Arg Gly Asp Arg Val Trp Gln Ile Ala Phe Gly Ser Gly Phe Lys Cys  
 450 455 460  
 Asn Ser Ala Val Trp Lys Cys Asn Arg Thr Ile Lys Thr Pro Lys Asp  
 465 470 475 480  
 Gly Pro Trp Ser Asp Cys Ile Asp Arg Tyr Pro Val Phe Ile Pro Glu  
 485 490 495  
 Val Val Lys Leu  
 500

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1548 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: Genomic DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATGGACGGTG	CCGAGAATC	ACGACTCGGT	GGTGATGGTG	GTGGTGATGG	TTCTGTTGGA	60
GTTTCAGATCC	GACAAACACG	GATGCTACCG	GATTTTCTCC	AGAGCGTGAA	TCTCAAGTAT	120
GTGAAATTAG	GTTACCATT	CTTAATCTCA	AATCTCTTGA	CTCTCTGTTT	ATTCCCTCTC	180
GCCGTTGTTA	TCTCCGTCGA	AGCCTCTCAG	ATGAACCCAG	ATGATCTCAA	ACAGCTCTGG	240
ATCCATCTAC	AATACAATCT	GGTTAGTATC	ATCATCTGTT	CAGCGATTCT	AGTCTTCGGG	300
TTAACGGTTT	ATGTTATGAC	CCGACCTAGA	CCCCTTACT	TGGTTGATTT	CTCTGTTTAT	360
CTCCACCTG	ATCATCTCAA	AGCTCCTTAC	GCTCGGTTCA	TGGAACATTC	TAGACTCACC	420
GGAGATTTCG	ATGACTCTGC	TCTCGAGTTT	CAACGCAAGA	TCCTTGAGCG	TTCTGGTTTA	480
GGGGAAGACA	CTTATGTCCC	TGAAGCTATG	CATTATGTTT	CACCGAGAAT	TTCAATGGCT	540
GCTGCTAGAG	AAGAAGCTGA	ACAAGTCATG	TTTGGTGCTT	TAGATAACCT	TTTCGCTAAC	600
ACTAATGTGA	AACCAAAGGA	TATTGGAATC	CTTGTTGTGA	ATTGTAGTCT	CTTTAATCCA	660
ACTCCTTCGT	TATCTGCAAT	GATTGTGAAC	AAGTATAAGC	TTAGAGGTAA	CATTAGAAGC	720
TACAATCTAG	GCGGTATGGG	TTGCAGCGCG	GGAGTTATCG	CTGTGGATCT	TGCTAAAGAC	780
ATGTTGTTGG	TACATAGGAA	CACTTATGCG	GTTGTTGTTT	CTACTGAGAA	CATTACTCAG	840
AATTGGTATT	TTGGTAACAA	GAAATCGATG	TTGATACCGA	ACTGCTTGTT	TCGAGTTGGT	900
GGCTCTGCGG	TTTTGCTATC	GAACAAGTCG	AGGGACAAGA	GACGGTCTAA	GTACAGGCTT	960
GTACATGTAG	TCAGGACTCA	CCGTGGAGCA	GATGATAAAG	CTTTCCGTTG	TGTTTATCAA	1020
GAGCAGGATG	ATACAGGGAG	AACCGGGGTT	TCGTGTGCGA	AAGATCTAAT	GGCGATTGCA	1080
GGGGAAACTC	TCAAAACCAA	TATCACTACA	TTGGGTCCTC	TTGTTCTACC	GATAAGTGAG	1140
CAGATTCTCT	TCTTTATGAC	TCTAGTTGTG	AAGAAGCTCT	TTAACGGTAA	AGTGAAACCG	1200
TATATCCCGG	ATTTCAAAC	TGCTTTCGAG	CATTTCTGTA	TCCATGCTGG	TGGAAGAGCT	1260
GTGATCGATG	AGTTAGAGAA	GAATCTGCAG	CTTTCACCAG	TTCATGTCGA	GGCTTCGAGG	1320
ATGACTCTTC	ATCGATTGGG	TAACACATCT	TCGAGCTCCA	TTTGGTATGA	ATTGGCTTAC	1380
ATTGAAGCGA	AGGGAAGGAT	GCGAAGAGGT	AATCGTGTTT	GGCAAATCGC	GTTCCGGAAGT	1440
GGATTAAAT	GTAATAGCGC	GATTTGGGAA	GCATTAAGGC	ATGTGAAACC	TTTCAACAAC	1500
AGTCCTTGGG	AAGATTGTAT	TGACAAGTAT	CCGGTAAC	TAAGTTAT		1548

## (2) INFORMATION FOR SEQ ID NO:14:

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## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 516 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```

Met Asp Gly Ala Gly Glu Ser Arg Leu Gly Gly Asp Gly Gly Gly Asp
 1      5      10      15
Gly Ser Val Gly Val Gln Ile Arg Gln Thr Arg Met Leu Pro Asp Phe
 20      25      30
Leu Gln Ser Val Asn Leu Lys Tyr Val Lys Leu Gly Tyr His Tyr Leu
 35      40      45
Ile Ser Asn Leu Leu Thr Leu Cys Leu Phe Pro Leu Ala Val Val Ile
 50      55      60
Ser Val Glu Ala Ser Gln Met Asn Pro Asp Asp Leu Lys Gln Leu Trp
 65      70      75      80
Ile His Leu Gln Tyr Asn Leu Val Ser Ile Ile Ile Cys Ser Ala Ile
 85      90      95
Leu Val Phe Gly Leu Thr Val Tyr Val Met Thr Arg Pro Arg Pro Val
100      105      110
Tyr Leu Val Asp Phe Ser Cys Tyr Leu Pro Pro Asp His Leu Lys Ala
115      120      125
Pro Tyr Ala Arg Phe Met Glu His Ser Arg Leu Thr Gly Asp Phe Asp
130      135      140
Asp Ser Ala Leu Glu Phe Gln Arg Lys Ile Leu Glu Arg Ser Gly Leu
145      150      155      160
Gly Glu Asp Thr Tyr Val Pro Glu Ala Met His Tyr Val Pro Pro Arg
165      170      175
Ile Ser Met Ala Ala Ala Arg Glu Glu Ala Glu Gln Val Met Phe Gly
180      185      190
Ala Leu Asp Asn Leu Phe Ala Asn Thr Asn Val Lys Pro Lys Asp Ile
195      200      205
Gly Ile Leu Val Val Asn Cys Ser Leu Phe Asn Pro Thr Pro Ser Leu
210      215      220
Ser Ala Met Ile Val Asn Lys Tyr Lys Leu Arg Gly Asn Ile Arg Ser
225      230      235      240
Tyr Asn Leu Gly Gly Met Gly Cys Ser Ala Gly Val Ile Ala Val Asp
245      250      255
Leu Ala Lys Asp Met Leu Leu Val His Arg Asn Thr Tyr Ala Val Val
260      265      270
Val Ser Thr Glu Asn Ile Thr Gln Asn Trp Tyr Phe Gly Asn Lys Lys
275      280      285
Ser Met Leu Ile Pro Asn Cys Leu Phe Arg Val Gly Gly Ser Ala Val
290      295      300
Leu Leu Ser Asn Lys Ser Arg Asp Lys Arg Arg Ser Lys Tyr Arg Leu
305      310      315      320
Val His Val Val Arg Thr His Arg Gly Ala Asp Asp Lys Ala Phe Arg
325      330      335
Cys Val Tyr Gln Glu Gln Asp Asp Thr Gly Arg Thr Gly Val Ser Leu
340      345      350
Ser Lys Asp Leu Met Ala Ile Ala Gly Glu Thr Leu Lys Thr Asn Ile
355      360      365
Thr Thr Leu Gly Pro Leu Val Leu Pro Ile Ser Glu Gln Ile Leu Phe
370      375      380
Phe Met Thr Leu Val Val Lys Lys Leu Phe Asn Gly Lys Val Lys Pro
385      390      395      400
Tyr Ile Pro Asp Phe Lys Leu Ala Phe Glu His Phe Cys Ile His Ala
405      410      415
Gly Gly Arg Ala Val Ile Asp Glu Leu Glu Lys Asn Leu Gln Leu Ser
420      425      430
Pro Val His Val Glu Ala Ser Arg Met Thr Leu His Arg Phe Gly Asn
435      440      445

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WHAT IS CLAIMED IS:

1. An isolated polynucleotide encoding a polypeptide having an amino acid sequence selected from the group consisting of: an amino acid sequence substantially identical to SEQ ID NO:2, an amino acid sequence substantially identical to SEQ ID NO:4, an amino acid sequence substantially identical to SEQ ID NO:6, an amino acid sequence substantially identical to SEQ ID NO:8, an amino acid sequence substantially identical to SEQ ID NO:10, an amino acid sequence substantially identical to SEQ ID NO:12, and an amino acid sequence substantially identical to SEQ ID NO:14.
2. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:2.
3. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:4.
4. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:6.
5. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:8.
6. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:10.
7. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:12.
8. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:14.

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9. An isolated polynucleotide, wherein said polynucleotide is selected from the group consisting of:

- a) SEQ ID NO:1;
- b) SEQ ID NO:3;
- c) SEQ ID NO:5;
- d) SEQ ID NO:7;
- e) SEQ ID NO:9;
- f) SEQ ID NO:11;
- g) SEQ ID NO:13;
- h) an RNA analog of SEQ ID NO:1;
- i) an RNA analog of SEQ ID NO:3;
- j) an RNA analog of SEQ ID NO:5;
- k) an RNA analog of SEQ ID NO:7;
- l) an RNA analog of SEQ ID NO:9;
- m) an RNA analog of SEQ ID NO:11;
- n) an RNA analog of SEQ ID NO:13;
- o) a polynucleotide having a nucleic acid sequence complementary to a), b), c), d), e), f), g), h), i), j), k), l), m), or n); and
- p) a nucleic acid fragment of a), b), c), d), e), f), g), h), i), j), k), l), m), n), or o) that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14.

10. An isolated polypeptide having an amino acid sequence selected from the group consisting of: an amino acid sequence substantially identical to SEQ ID NO:2, an amino acid sequence substantially identical to SEQ ID NO:4, an amino acid sequence substantially identical to SEQ ID NO:6, an amino acid sequence substantially identical to SEQ ID NO:8, an amino acid sequence substantially identical to SEQ ID NO:10, an amino acid sequence substantially identical to SEQ ID NO:12, and an

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amino acid sequence substantially identical to SEQ ID NO:14.

11. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:2.

12. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:4.

13. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:6.

14. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:8.

15. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:10.

16. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:12.

17. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:14.

18. A transgenic plant containing a nucleic acid construct comprising a polynucleotide selected from the group consisting of:

- a) SEQ ID NO:1;
- b) SEQ ID NO:3;
- c) SEQ ID NO:5;
- d) SEQ ID NO:7;
- e) SEQ ID NO:9;
- f) SEQ ID NO:11;
- g) SEQ ID NO:13;
- h) an RNA analog of SEQ ID NO:1;

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- i) an RNA analog of SEQ ID NO:3;
- j) an RNA analog of SEQ ID NO:5;
- k) an RNA analog of SEQ ID NO:7;
- l) an RNA analog of SEQ ID NO:9;
- m) an RNA analog of SEQ ID NO:11;
- n) an RNA analog of SEQ ID NO:13;
- o) a polynucleotide having a nucleic acid sequence complementary to a), b), c), d), e), f), g), h), i), j), k), l), m), or n); and
- p) a nucleic acid fragment of a), b), c), d), e), f), g), h), i), j), k), l), m), n), or o) that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14.

19. The plant of claim 18, wherein said construct further comprises a regulatory element operably linked to said polynucleotide.

20. The plant of claim 19, wherein said regulatory element is a tissue-specific promoter.

21. The plant of claim 20, wherein said regulatory element is an epidermal cell-specific promoter.

22. The plant of claim 20, wherein said regulatory element is a seed-specific promoter that is operably linked in sense orientation to said polynucleotide.

23. The plant of claim 22, wherein said plant has altered levels of very long chain fatty acids in seeds compared to the levels in a plant lacking said nucleic acid construct.



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24. A transgenic plant containing a nucleic acid construct comprising a polynucleotide encoding a polypeptide selected from the group consisting of: an amino acid sequence substantially identical to SEQ ID NO:2, an amino acid sequence substantially identical to SEQ ID NO:4, an amino acid sequence substantially identical to SEQ ID NO:6, an amino acid sequence substantially identical to SEQ ID NO:8, an amino acid sequence substantially identical to SEQ ID NO:10, an amino acid sequence substantially identical to SEQ ID NO:12, and an amino acid sequence substantially identical to SEQ ID NO:14.

25. The plant of claim 24, wherein said construct further comprises a regulatory element operably linked to said polynucleotide.

26. The plant of claim 25, wherein said regulatory element is a tissue-specific promoter.

27. The plant of claim 26, wherein said regulatory element is an epidermal cell-specific promoter.

28. The plant of claim 26, wherein said regulatory element is a seed-specific promoter that is operably linked in sense orientation to said polynucleotide.

29. The plant of claim 28, wherein said plant has altered levels of very long chain fatty acids in seeds compared to the levels in a plant lacking said nucleic acid construct.

30. A method of altering the levels of very long chain fatty acids in a plant, comprising the steps of:

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A) creating a nucleic acid construct, said construct comprising a polynucleotide selected from the group consisting of:

- a) SEQ ID NO:1;
  - b) SEQ ID NO:3;
  - c) SEQ ID NO:5;
  - d) SEQ ID NO:7;
  - e) SEQ ID NO:9;
  - f) SEQ ID NO:11;
  - g) SEQ ID NO:13;
  - h) an RNA analog of SEQ ID NO:1;
  - i) an RNA analog of SEQ ID NO:3;
  - j) an RNA analog of SEQ ID NO:5;
  - k) an RNA analog of SEQ ID NO:7;
  - l) an RNA analog of SEQ ID NO:9;
  - m) an RNA analog of SEQ ID NO:11;
  - n) an RNA analog of SEQ ID NO:13;
  - o) a polynucleotide having a nucleic acid sequence complementary to a), b), c), d), e), f), g), h), i), j), k), l), m), or n); and
  - p) a nucleic acid fragment of a), b), c), d), e), f), g), h), i), j), k), l), m), n), or o) that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14; and
- B) introducing said construct into said plant, wherein said polynucleotide is effective for altering the levels of very long chain fatty acids in said plant.

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Figure 1

FAEl w/ respect to time

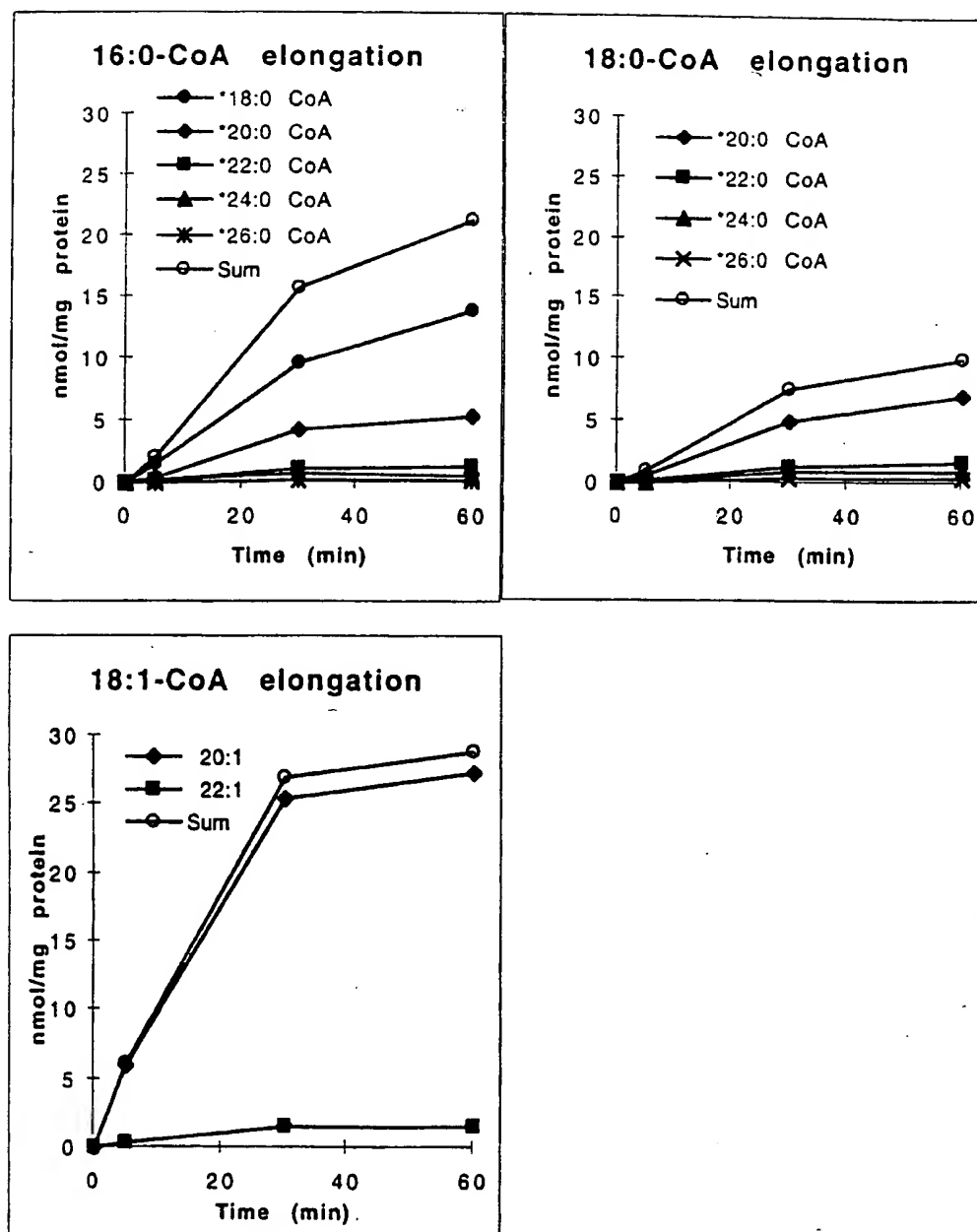
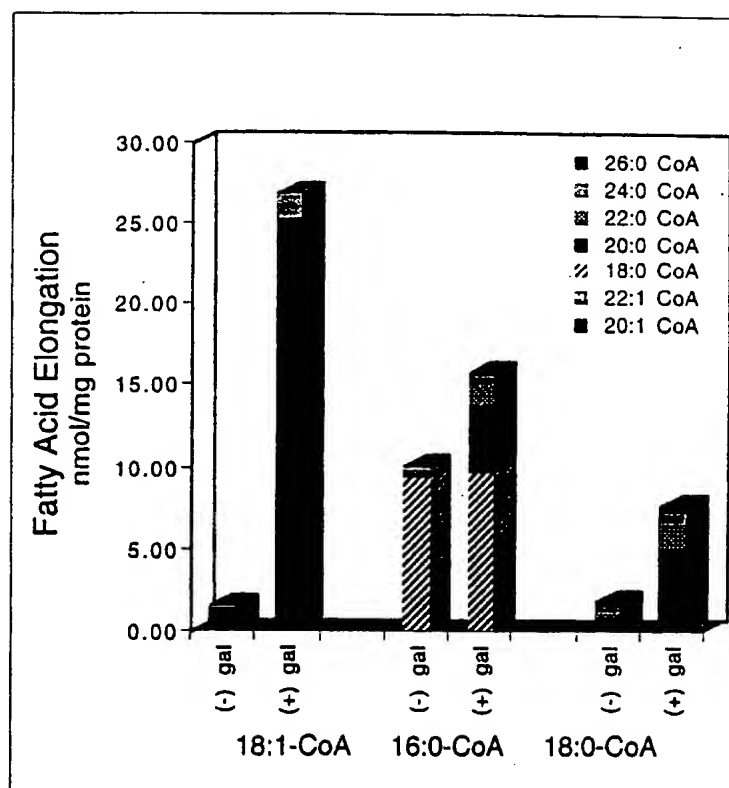


Figure 2



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EL1 1560 bases

ATGGATCGAG	AGAGATTAAC	GGCGGAGATG	GCGTTTCGAG	ATTCATCATC	GGCCGTTATA
AGAATTCGAA	GACGTTTGCC	GGATTTATTA	ACGTCCGTTA	AGCTCAAATA	CGTGAAGCTT
GGACTTCACA	ACTCTTGCAA	CGTGACCACC	ATTCTCTTCT	TCTTAATTAT	TCTTCCTTTA
ACCGGAACCG	TGCTGGTTCA	GCTAACCGGT	CTAACGTTCT	ATACGTTCTC	TGAGCTTTGG
TCTAACCAGG	CGGTTCAACT	CGACACGGCG	ACGAGACTTA	CCTGCTTGGT	TTTCCCTCTCC
TTTCGTTTTGA	CCCTCTACGT	GGCTAACCGG	TCTAAACCGG	TTTACCTAGT	GGATTTCTCC
TGCTACAAAC	CGGAAGACGA	GCGTAAAATA	TCAGTAGATT	CGTTCTTGAC	GATGACTGAG
GAAAATGGAT	CATTCAACCGA	TGACACGGTT	CAGTTCCAGC	AAAGAATCTC	GAACCGGGCC
GGTTTGGGAG	ACGAGACGTA	TCTGCCACGT	GGCATAACTT	CAACGCCCCC	GAAGCTAAAT
ATGTCAGAGG	CACGTGCCGA	AGCTGAAGCC	GTTATGTTTG	GAGCCTTAGA	TTCCCTCTTC
GAGAAAACCG	GAATTAAACC	GGCCGAAGTC	GGAATCTTGA	TAGTAAACTG	CAGCTTATTC
AATCCGACGC	CGTCTCTATC	AGCGATGATC	GTGAACCATT	ACAAGATGAG	AGAAGACATC
AAAAGTTACA	ACCTCGGAGG	AATGGGTTGC	TCCGCCGGAT	TAATCTCAAT	CGATCTCGCT
AACAATCTCC	TCAAAGCAAA	CCCTAATTCT	TACGCTGTCT	TGGTAAGCAC	GGAAAACATA
ACCCTAAACT	GGTACTTCGG	AAATGACCGG	TCAATGCTCC	TCTGCAACTG	CATCTTCCGA
ATGGGCGGAG	CTGCGATTCT	CCTCTCTAAC	CGCCGTCAAG	ACCGGAAGAA	GTCAAAGTAC
TCGCTGGTCA	ACGTCGTTCT	AACACATAAA	GGATCAGACG	ACAAGAACTA	CAATTGCGTG
TACCAGAAGG	AAGACGAGAG	AGGAACAATC	GGTGTCTCTT	TAGCTAGAGA	GCTCATGTCT
GTCGCCGGAG	ACGCTCTGAA	AACAAACATC	ACGACTTTAG	GACCGATGGT	TCTTCCATTG
TCAGAGCAGT	TGATGTTCTT	GATTTCCCTG	GTCAAAAGGA	AGATGTTCAA	GTTAAAAGTT
AAACCGTATA	TTCCGGATTT	CAAGCTAGCT	TTCGAGCATT	TCTGTATTCA	CGCAGGAGGT
AGAGCGGTTT	TAGACGAAGT	GCAGAAGAAT	CTTGATCTCA	AAGATTGGCA	CATGGAACCT
TCTAGAATGA	CTTTGCACAG	ATTTGGTAAC	ACTTCGAGTA	GCTCGCTTTG	GTATGAGATG
GCTTATACCG	AAGCTAAGGG	TCGGGTTAAA	GCTGGTGACC	GACTTTGGCA	GATTGCGTTT
GGATCGGGTT	TCAAGTGTA	TAGTGCGGTT	TGGAAAGCGT	TACGACCGGT	TTCCGACGGAG
GAGATGACCG	GTAATGCTTG	GGCTGGTTCT	ATTGATCAAT	ATCCGGTTAA	AGTTGTGCAA

EL1  
FIGURE 3

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## EL1 sequence

Molecular Weight 58379.00 Daltons

520 Amino Acids

62 Strongly Basic(+) Amino Acids (K,R)

52 Strongly Acidic(-) Amino Acids (D,E)

187 Hydrophobic Amino Acids (A,I,L,F,W,V)

144 Polar Amino Acids (N,C,Q,S,T,Y)

8.784 Isoelectric Point

10.804 Charge at PH 7.0

MDRERLTAEM	AFRDSSSAVI	RIRRRLPDLL	TSVKLKYVKL	GLHNSCNVTT	ILFFLIILPL
TGTVLVQLTG	LTFDTFSELW	SNQAVQLDTA	TRLTCLVFLS	FVLTLYVANR	SKPVYLVDFS
CYKPEDERKI	SVDSFLTMTE	ENGSTDDTV	QFQQRISNRA	GLGDETYLPR	GITSTPPKLN
MSEARAEAEA	VMFGALDSL	EKTGIKPAEV	GILIVNCSLF	NPTPSLSAMI	VNHYKMREDI
KSYNLGGMGC	SAGLISIDLA	NNLLKANPNS	YAVVVSTENI	TLNWYFGNDR	SMLLCNCIFR
MGGAAILLN	RRQDRKKSKY	SLVNVVRTHK	GSDDKNYNVCV	YQKEDERGTI	GVSLARELMS
VAGDALKTNI	TTLGPMVLPL	SEQLMFLISL	VKRKMFCLKV	KPYIPDFKLA	FEHFCIHAGG
RAVLDEVQKN	LDLKDWHMEP	SRMTLHRFGN	TSSSSLWYEM	AYTEAKGRVK	AGDRLWQIAF
GSGFKCNSAV	WKALRPVSTE	EMTGNAWAGS	IDQYPVKVVQ		

FIGURE 4

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EL2 1479 bases

ATGGATTACC	CCATGAAGAA	GGTAAAAATC	TTTTTCAACT	ACCTCATGGC	GCATCGCTTC	
AAGCTCTGCT	TCTTACCATT	AATGGTTGCT	ATAGCCGTGG	AGGCGTCTCG	TCTTTCCACA	120
CAAGATCTCC	AAAACCTTTA	CCTCTACTTA	CAAAACAACC	ACACATCTCT	AACCATGTTC	
TTCTTTTACC	TCGCTCTCGG	GTCGACTCTT	TACCTCATGA	CCCGGCCCAA	ACCCGTTTAT	240
CTCGTTGACT	TTAGCTGCTA	CCTCCCACCG	TCGCATCTCA	AAGCCAGCAC	CCAGAGGATC	
ATGCAACACG	TAAGGCTTGT	ACGAGAAGCA	GGCGCGTGGA	AGCAAGAGTC	CGATTACTTG	360
ATGGACTTCT	GCGAGAAGAT	TCTAGAACGT	TCCGGTCTAG	GCCAAGAGAC	GTACGTACCC	
GAAGGTCTTC	AACTTTTGCC	ACTACAACAG	AATTTGGCTG	TATCACGTAT	AGAGACGGAG	480
GAAGTTATTA	TTGGTGCGGT	CGATAATCTG	TTTCGCAACA	CGGGAATAAG	CCCTAGTGAT	
ATAGGTATAT	TGGTGGTGAA	TTCAAGCACT	TTTAATCCAA	CACCTTCGCT	ATCAAGTATC	600
TTAGTGAATA	AGTTTAAACT	TAGGGATAAT	ATAAAGAGCT	TGAATCTTGG	TGGGATGGGG	
TGTAGCGCTG	GAGTCATCGC	TATCGATGCG	GCTAAGAGCT	TGTTACAAGT	TCATAGAAAC	720
ACTTATGCTC	TTGTGGTGAG	CACGGAGAAC	ATCACTCAAA	ACTTGTACAT	GGGTAACAAC	
AAATCAATGT	TGGTTACAAA	CTGTTTGTTT	CGTATAGGTG	GGGCCGCGAT	TTTGCTTTCT	840
AACCGGTCTA	TAGATCGTAA	ACGCGCAAAA	TACGAGCTTG	TTACACCCGT	GCGGGTCCAT	
ACCGGAGCAG	ATGACCGATC	CTATGAATGT	GCAACTCAAG	AAGAGGATGA	AGATGGCATA	960
GTTGGGGTTT	CCTTGTCAAA	GAATCTACCA	ATGGTAGCTG	CAAGAACCCT	AAAGATCAAT	
ATCGCAACTT	TGGGTCCGCT	TGTTCTTCCC	ATAAGCGAGA	AGTTTCACTT	CTTTGTGAGG	1080
TTCTGTTAAAA	AGAAGTTTCT	CAACCCCAAG	CTAAAGCATT	ACATTCCGGA	TTTCAAGCTC	
GCATTTCGAGC	ATTTCTGTAT	CCATGCGGGT	GGTAGAGCGC	TAATTGATGA	GATGGAGAAG	1200
AATCTTCATC	TAACTCCACT	AGACGTTGAG	GCTTCAAGAA	TGACATTACA	CAGGTTTGGT	
AATACCTCTT	CGAGCTCCAT	TTGGTACGAG	TTGGCTTACA	CAGAAGCCAA	AGGAAGGATG	1320
ACGAAAGGAG	ATAGGATTTG	GCAGATTGCG	TTGGGGTCAG	GTTTTAAGTG	TAATAGTTCA	
GTTTGGGTGG	CTCTTCGTAA	CGTCAAGCCT	TCTACTAATA	ATCCTTGGA	ACAGTGTCTA	1440
CACAAATATC	CAGTTGAGAT	CGATATAGAT	TTAAAAGAG			

EL2  
FIGURE 5

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EL2 protein sequence  
Molecular Weight 55799.30 Daltons  
493 Amino Acids  
55 Strongly Basic(+) Amino Acids (K,R)  
46 Strongly Acidic(-) Amino Acids (D,E)  
181 Hydrophobic Amino Acids (A,I,L,F,W,V)  
134 Polar Amino Acids (N,C,Q,S,T,Y)  
8.756 Isoelectric Point  
10.995 Charge at PH 7.0

MDYPMKKVKI FFNYLMAHRF KLCFLPLMVA IAVEASRLST QDLQNFYLYL QNNHTSLTMF FLYLALGSTL  
YLMTRPKPVY LVDFSCYLPP SHLKASTQRI MQHVRLVREA GAWKQESDYL MDFCEKILER SGLGQETYVP  
EGLQTLPLQQ NLAVSRIETE EVIIGAVDNL FRNTGISPSD IGILVVNSST FNPTPSLSSI LVNKFKLRDN  
IKSLNLGGMG CSAGVIAIDA AKSLLQVHRN TYALVVSTEN ITQNLVYGNN KSMLVTNCLF RIGGAAILLS  
NRSIDRKRAK YELVHTVRVH TGADDRSYEC ATQEEDEDGI VGVSLSKNLP MVAARTLKIN IATLGPLVLP  
ISEKFHFFVR FVKKKFLNPK LKHYIPDFKL AFEHFCIHAG GRALIDEMEK NLHLTPLDVE ASRMTLHRFG  
NTSSSSIWYE LAYTEAKGRM TKGDRIWQIA LGSGFKCNSS VWVALRNVKP STNNPWEQCL HKYPVEIDID  
LKE

FIGURE 6



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EL3 1512 bases

CTACGTCAGG	GTAGAACAAA	GAGTAAACAC	TTAAGCAAAA	CAATTGTGCC	TACTCTTAGG	TTATCTCCAA
TGAAGAACTT	AAAGATGGTT	TTCTTCAAGA	TCCTCTTTAT	CTCTTTAATG	GCAGGATTAG	CCATGAAAGG
ATCTAAGATC	AACGTAGAAG	ATCTCCAAAA	GTTCTCCCTC	CACCATACAC	AGAACAACCT	CCAAACCATA
AGCCTTCTAT	TGTTTCTTGT	CGTTTTTGTG	TGGATCCTCT	ACATGTTAAC	CCGACCTAAA	CCCGTTTACC
TTGTTGATTT	CTCCTGCTAC	CTTCCACCGT	CGCATCTCAA	GGTCAGTATC	CAAACCCTAA	TGGGACACGC
AAGACGTGCA	AGAGAAGCAG	GCATGTGTTG	GAAGAACAAA	GAGAGCGACC	ATTTAGTTGA	CTTCCAGGAG
AAGATTCTTG	AACGTTCCGG	TCTTGGTCAA	GAAACCTACA	TCCCCGAGGG	TCTTCAGTGC	TTCCCACTTC
AGCAAGGCAT	GGGTGCTTCA	CGTAAAGAGA	CGGAAGAAAGT	AATCTTCGGA	GCTCTTGACA	ATCTTTTTTCG
CAACACCGGT	GTAAAACCTG	ATGATATCGG	TATATTGGTG	GTGAATTCTA	GCACGTTTAA	TCCAACCTCA
TCACTCGCCT	CCATGATTGT	GAACAAGTAC	AAACTCAGAG	ACAACATCAA	GAGTTTGAAT	CTTGGAGGGA
TGGGTTGCAG	TGCCGGAGTT	ATAGCTGTTG	ATGTCGCTAA	GGGATTACTA	CAAGTTCATA	GGAACTTCA
TGCTATTGTA	GTAAGCACAG	AGAACATCAC	TCAGAACTTA	TACTTGGGGA	AAAACAAATC	AATGCTAGTC
ACAAACTGTT	TGTCCGCGT	TGGTGGTGCT	GCGGTTCTGC	TTTCAAACAG	ATCTAGAGAC	CGTAACCGCG
CCAAATACGA	GCTTGTTTAC	ACCGTACGGA	TCCATACCGG	ATCAGATGAT	AGGTCGTTTCG	AATGTGCGAC
ACAAGAAGAG	GATGAAGATG	GTATAATTGG	AGTTACCTTG	ACAAAGAATC	TACCTATGGT	GGCTGCAAGG
ACTCTTAAGA	TAAATATCGC	AACTTTGGGT	CCTCTTGTAC	TTCCATTAAA	AGAGAAGCTA	GCCTTCTTTA
TTACTTTTGT	CAAGAAGAAG	TATTTCAAGC	CAGAGTTAAG	GAATTATACA	CCAGATTTCA	AGCTTGCCCTT
TGAGCATTTT	TGTATCCACG	CTGGTGGAAG	AGCTCTAATA	GATGAGCTGG	AGAAGAACCT	TAAGCTTTCT
CCGTTACACG	TAGAGGCGTC	AAGAATGACA	CTACACAGGT	TTGGTAACAC	TTCTTCTAGC	TCAATCTGGT
ACGAGTTAGC	TTATACAGAA	GCTAAAGGAA	GGATGAAGGA	AGGAGATAGG	ATTTGGCAGA	TTGCTTTGGG
GTCAGGTTTT	AAGTGTAACA	GTTCAGTATG	GGTGGCTCTG	CGAGACGTTA	AGCCTTCAGC	TAACAGTCCA
TGGGAAGACT	GTATGGATAG	ATATCCGGTT	GAGATTGATA	TT		

EL3  
FIGURE 7

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## EL3 protein sequence

Molecular Weight 56801.10 Daltons

504 Amino Acids

66 Strongly Basic(+) Amino Acids (K,R)

48 Strongly Acidic(-) Amino Acids (D,E)

183 Hydrophobic Amino Acids (A,I,L,F,W,V)

127 Polar Amino Acids (N,C,Q,S,T,Y)

9.315 Isoelectric Point

19.797 Charge at PH 7.0

LRQGRTKSKH LSKTICPTLR LSPMKNLKMV FFKILFISLM AGLAMKGSKI NVEDLQKFSL HHTQNNLQTI  
SLLLFLVVVFV WILYMLTRPK PVYLVDFSCY LPPSHLKVSI QTLMGHARRA REAGMCWKNK ESDHLVDFQE  
KILERSGLGQ ETYIPEGLQC FPLQQGMGAS RKETEIVIFG ALDNLFRNTG VKPDDIGILV VNSSTFNPTP  
SLASMIYNKY KLRDNIKSLN LGGMGCSAGV IAVDVAKGLL QVHRNTYAIV VSTENITQNL YLGKNKSMMLV  
TNCLFRVGGG AVLLSNRSRD RNRKYELVH TVRIHTGSDD RSFECATQEE DEDGIIIGVTL TKNLPMVAAR  
TLKINIATLG PLVLPLKEKL AFFITFVKKK YFKPELRNYT PDFKLAFEHF CIHAGGRALI DELEKNLKLKLS  
PLHVEASRMT LHRFGNTSSS SIWYELAYTE AKGRMKEGDR IWQIALGSGF KCNSSVWVAL RDVKPSANSF  
WEDCMDRYPV EIDI

EL3

FIGURE 8

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EL4 cDNA 1650 bases

ATGGGTTAGAT	CCAACGAGCA	AGATCTGCTC	TCTACCGAGA	TCGTTAATCG	TGGGATCGAA	CCATCCGGTC
CTAACGCCGG	CTCACCAACG	TTCTCGGTTA	GGGTCAGGAG	ACGTTTGCCT	GATTTTCTTC	AGTCGGTGAA
CTTGAAGTAC	GTGAAACTTG	GTTACCACTA	CCTCATAAAC	CATGCGGTTT	ATTTGGCGAC	CATACCGGTT
CTTGTGCTGG	TTTTTAGTGC	TGAGGTTGGG	AGTTTAAAGCA	GAGAAGAGAT	TTGGAAGAAG	CTTTGGGACT
ATGATCTTGC	AACTGTTATC	GGATTCTTCG	GTGTCTTTGT	TTTAACCGCT	TGTGTCTACT	TCATGTCTCG
TCCTCGCTCT	GTTTATCTTA	TTGATTTTCG	TTGTTACAAG	CCCTCCGATG	AACACAAGGT	GACAAAAGAA
GAGTTCATAG	AACTAGCGAG	AAAATCAGGG	AAGTTCGACG	AAGAGACACT	CGGTTTCAAG	AAGAGGATCT
TACAAGCCTC	AGGCATAGGC	GACGAGACAT	ACGTCCCAAG	ATCCATCTCT	TCATCAGAAA	ACATAACAAC
GATGAAAGAA	GGTCGTGAAG	AAGCCTCTAC	AGTGATCTTT	GGAGCACTAG	ACGAACTCTT	CGAGAAGACA
CGTGTAAGAA	CTAAAGACGT	TGGTGTCTCT	GTGGTTAACT	GTAGCATTTT	CAACCCGACA	CCGTCGTTGT
CCGCAATGGT	GATAAACCAT	TACAAGATGA	GAGGGAACAT	ACTTAGTTAC	AACCTTGGAG	GGATGGGATG
TTCGGCTGGA	ATCATAGCTA	TTGATCTTGC	TCGTGACATG	CTTCAGTCTA	ACCCTAATAG	TTATGCTGTT
GTTGTGAGTA	CTGAGATGGT	TGGGTATAAT	TGGTACGTGG	GAAGTGACAA	GTCAATGGTT	ATACCTAATT
GTTTCTTTAG	GATGGGTTGT	TCTGCCGTTA	TGCTCTCTAA	CCGTCGTCGT	GACTTTCGCC	ATGCTAAGTA
CCGTCTCGAG	CACATTGTCC	GAATCATAA	GGCTGCTGAC	GACCGTAGCT	TCAGGAGTGT	GTACCAGGAA
GAAGATGAAC	AAGGATTCAA	GGGGTTGAAG	ATAAGTAGAG	ACTTAATGGA	AGTTGGAGGT	GAAGCTCTCA
AGACAAACAT	CACTACCTTA	GGTCCTCTTG	TCCTACCTTT	CTCCGAGCAG	CTTCTCTTCT	TTGCTGCTTT
GGTCCGCCGA	ACATTCTCAC	CTGCTGCCAA	AACGTCCACA	ACCACTTCCT	TCTCTACTTC	CGCCACCGCA
AAAACCAATG	GAATCAAGTC	TTCTCTTCC	GATCTGTCCA	AGCCATACAT	CCCGGACTAC	AAGCTCGCCT
TCGAGCATTT	TTGCTTCCAC	GCGGCAAGCA	AAGTAGTGCT	TGAAGAGCTT	CAAAAGAATC	TAGGCTTGAG
TGAAGAGAAT	ATGGAGGCTT	CTAGGATGAC	ACTTCACAGG	TTTGGAACA	CTTCTAGCAG	TGGAATCTGG
TATGAGTTGG	CTTACATGGA	GGCCAAGGAA	AGTGTTCGTA	GAGGCGATAG	GGTTTGGCAG	ATCGCTTTCG
GTTCTGGTTT	TAAGTGTAAC	AGTGTGGTGT	GGAAGGCAAT	GAGGAAGGTG	AAGAAGCCAA	CCAGGAACAA
TCCTTGGGTG	GATTGCATCA	ACCGTTACCC	TGTGCCTCTC			

EL4  
FIGURE 9

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EL4 protein sequence  
Molecular Weight 61953.80 Daltons  
550 Amino Acids  
71 Strongly Basic(+) Amino Acids (K,R)  
58 Strongly Acidic(-) Amino Acids (D,E)  
191 Hydrophobic Amino Acids (A,I,L,F,W,V)  
147 Polar Amino Acids (N,C,Q,S,T,Y)  
9.036 Isoelectric Point  
14.349 Charge at PH 7.0

MGRSNEQDLL	STEIVNRGIE	PSGPNAGSPT	FSVRVRRRLP	DFLQSVNLKY	VKLGYHYLIN	HAVYLATIPV
LVLVFSAEVG	SLSREEIWKK	LWDYDLATVI	GFFGVFVLTA	CVYFMSRPRS	VYLIDFACYK	PSDEHKVTKE
EFIELARKSG	KFDEETLGFK	KRILQASGIG	DETYVPRIS	SSENITTMKE	GREEASTVIF	GALDELFEKT
RVKPKDVGVL	VVNCISIFNPT	PSLSAMVINH	YKMRGNILSY	NLGGMGCSAG	IIAIDLARDM	LQSNPNYSYAV
VVSTEMVGYN	WYVGSDKSMV	IPNCFFRMGC	SAVMLSNRRR	DFRHAKYRLE	HIVRTHKAAD	DRSFERSVYQE
EDEQGFKGLK	ISRDLMEVGG	EALKTNITTL	GPLVLPFSEQ	LLFFAALVRR	TFSPAAKTST	TTSFSTSATA
KTNGIKSSSS	DLSPYIPDY	KLAFEHFCFH	AASKVVLEEL	QKNLGLSEEN	MEASRMTLHR	FGNTSSSGIW
YELAYMEAKE	SVRRGDRVWQ	IAFGSGFKCN	SVVWKAMRKV	KKPTRNNPWV	DCINRYPVPL	

EL4  
FIGURE 10

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EL5 cDNA 1611 bases

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TCGAGCTACG TCAGGGCTTT TATATGCACA AATTCTCATA AAGTTTTCAA TTTTATTCCA TTTTCTCGG
AAGCCATGGA AGCTGCTAAT GAGCCTGTTA ATGGCGGATC CGTACAGATC CGAACAGAGA ACAACGAAAG
ACGAAAGCTT CCTAATTTCT TACAAAGCGT CAACATGAAA TACGTCAAGC TAGGTTATCA TTACCTCATT
ACTCATCTCT TCAAGCTCTG TTTGGTTCCA TTAATGGCGG TTTTAGTCAC AGAGATCTCT CGATTAACAA
CAGACGATCT TTACCAGATT TGGCTTCATC TCCAATACAA TCTCGTTGCT TTCATCTTTC TCTCTGCTTT
AGCTATCTTT GGCTCCACCG TTTACATCAT GAGTCGTCCC AGATCTGTTT ATCTCGTTGA TTACTCTTGT
TATCTTCCTC CGGAGAGTCT TCAGGTTAAG TATCAGAAGT TTATGGATCA TTCTAAGTTG ATTGAAGATT
TCAATGAGTC ATCTTTAGAG TTTCAGAGGA AGATTCTTGA ACGTTCTGGT TTAGGAGAAG AGACTTATCT
CCCTGAAGCT TTACATTGTA TCCCTCCGAG GCCTACGATG ATGGCGGCTC GTGAGGAATC TGAGCAGGTA
ATGTTTGGTG CTCTTGATAA GCTTTTCGAG AATACCAAGA TTAACCCTAG GGATATTGGT GTGTTGGTTG
TGAATTGTAG CTTGTTTAAAT CCTACACCTT CGTTGTCAGC TATGATTGTT AACAAGTATA AGCTTAGAGG
GAATGTTAAG AGTTTTAACC TTGGTGGAAT GGGGTGTAGT GCTGGTGTTA TCTCTATCGA TTTAGCTAAA
GATATGTGTC AAGTTCATAG GAATACTTAT GCTGTTGTGG TTAGTACTGA GAACATTACT CAGAATTGGT
ATTTTGGGAA TAAGAAGGCT ATGTTGATTC CGAATTGTTT GTTTCGTGTT GGTGGTTCGG CGATTTTGTT
GTCGAACAAG GGGAAAGATC GTAGACGGTC TAAGTATAAG CTTGTTCATA CCGTTAGGAC TCATAAAGGA
GCTGTTGAGA AGGCTTTCAA CTGTGTTTAC CAAGAGCAAG ATGATAATGG GAAGACCGGG GTTTCGTTGT
CGAAAGATCT TATGGCTATA GCTGGGGAAG CTCTTAAGGC GAATATCACT ACTTAGGTC CTTTGTTCT
TCCTATAAGT GAGCAGATTG TGTTTTTCAT GACTTTGGTT ACGAAGAAAC TGTTTAACTC GAAGCTGAAG
CCGTATATTC CGGATTTCAA GCTTGCGTTT GATCATTCTT GTATCCATGC TGGTGGTAGA GCTGTGATTG
ATGAGCTTGA GAAGAATCTG CAGCTTTCGC AGACTCATGT CGAGGCATCC AGAATGACAC TGCACAGATT
TGGAACACT TCTTCGAGCT CGATTTGGTA TGAAGTGGCT TACATAGAGG CTAAAGGTAG GATGAAGAAA
GGAAACCGGG TTTGGCAGAT TGCTTTTGA AGTGGGTTTA AGTGTAACAG TGCAGTTTGG GTGGCTCTAA
ACAATGTCAA GCCTTCGGTT AGTAGTCCGT GGGAAACACT CATCGACCGA TATCCGGTTA AGCTCGACTT
C

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EL5  
FIGURE 11

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EL5 protein sequence  
Molecular Weight 60874.60 Daltons  
537 Amino Acids  
63 Strongly Basic(+) Amino Acids (K,R)  
47 Strongly Acidic(-) Amino Acids (D,E)  
198 Hydrophobic Amino Acids (A,I,L,F,W,V)  
148 Polar Amino Acids (N,C,Q,S,T,Y)  
9.107 Isoelectric Point  
17.930 Charge at PH 7.0

SSYVRAFICT	NSHKVFNFIP	FFSEAMEAAN	EPVNGGSVQI	RTENNERRKL	PNFLQSVNMK	YVKLGYYHYLI
THLFLKCLVP	LMAVLVTEIS	RLTTDDLYQI	WLHLQYNLVA	FIFLSALAIF	GSTVYIMSRP	RSVYLVVDYSC
YLPPESLQVK	YQKFMDSKL	IEDFNESSLE	FQRKILERSG	LGEETYLPEA	LHCIPPRPTM	MAAREESEQV
MFGALDKLFE	NTKINPRDIG	VLVVNCSLFN	PTPSLSAMIV	NKYKLRGNVK	SFNLGGMGCS	AGVISIDLAK
DMLQVHRNTY	AVVVSTENIT	QNWYFGNKA	MLIPNCLFRV	GGSAILLSNK	GKDRRRSKYK	LVHTVRTHKG
AVEKAFNCVY	QEQDDNGKTG	VLSKDLMAI	AGEALKANIT	TLGPLVLPIS	EQILFFMTLV	TKKLFNSKLK
PYIPDFKLAF	DHFCIHAGGR	AVIDELEKNL	QLSQTHVEAS	RMTLHRFGNT	SSSSIWYELA	YIEAKGRMCK
GNRVWQIAFG	SGFKCNSAVW	VALNNVKPSV	SSPWEHCIDR	YPVKLDF		

EL5  
FIGURE 12

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EL6 1502 bases  
TCTCCGACGATGCCTCAGGCACCGATGCCAGAGTTCTCTAGCTCGGTGAAGCTCAAGTACGTGAAACTTGGTTACCAA  
TATTTGGTTAACCATTCTTGAGTTTTCTTTTGATCCCGATCATGGCTATTGTCGCCGTTGAGCTTCTTCGGATGGGT  
CCTGAAGAGATCCTTAATGTTTGGAAATCACTCCAGTTTGACCTAGTTCAGGTTCTATGTTCTTCCTTTTGTGATC  
TTCATCTCCACTGTTTACTTCATGTCCAAGCCACGCACCATCTACCTCGTTGACTATTCTTGTACAAAGCCACCTGTC  
ACGTGTCGTGTCCCCTTCGCAACTTTTCATGGAACACTCTCGTTTGATCCTCAAGGACAAGCCTAAGAGCGTCGAGTTC  
CAAATGAGAATCCTTGAACGTTCTGGCCTCGGTGAGGAGACTTGTCTCCCTCCGGCTATTCTTATATTCTCTCCACA  
CCAACCATGGACGCGGCTAGAAGCGAGGCTCAGATGGTTATCTTCGAGGCCATGGACGATCTTTCAAGAAAACCGGT  
CTTAAACCTAAAGACGTCGACATCCTTATCGTCAACTGCTCTCTTTTCTCTCCACACCATCGCTCTCAGCTATGGTC  
ATCAACAAATATAAGCTTAGGAGTAATATCAAGAGCTTCAATCTTTTCGGGGATGGGCTGCAGCGCGGGCCTGATCTCA  
GTTGATCTAGCCCGCGACTTGCTCCAAGTTCATCCCAATTCAAATGCAATCATCGTCAGCACGGAGATCATAACGCCT  
AATTACTATCAAGGCAACGAGAGAGCCATGTTGTTACCCAATTGTCCTTCCGCATGGGTGCGGCAGCCATACACATG  
TCAAACCGCCGCTCTGACCGGTGGCGAGCCAAATACAAGCTTTCCACCTCGTCCGGACACACCGTGGCGCTGACGAC  
AAGTCTTTCTACTGTGTCTACGAACAGGAAGACAAAGAAGGACACGTTGGCATCAACTTGTCCAAAGATCTCATGGCC  
ATCGCCGGTGAAGCCCTCAAGGCAAACATCACCACAATAGGTCCTTTGGTCCTACCGGCGTCAGAACAACCTTCTCTTC  
CTCAGCTCCCTAATCGGACGTAAATCTTCAACCCGAAATGGAAACCATAACCGGATTTCAAGCTGGCCTTCGAA  
CACTTTTGCATTACGCAGGAGGCAGAGCGGTGATCGACGAGCTCCAAAAGAATCTACAACATCAGGAGAACACGTT  
GAGGCCTCAAGAATGACACTACATCGTTTTGGTAACACGTCATCTTCATCGTTATGGTACGAGCTTAGCTACATCGAG  
TCTAAAGGGAGAATGAGGAGAGGCGATCGCGTTTGGCAAATCGCGTTTGGGAGTGGTTTCAAGTGTAACCTCTGCCGTG  
TGGAAGTGTAACCGTACGATTAAGACACCTAAGGACGGACCATGGTCCGATTGTATCGACCGTTACCCTGTCTTTATT  
CCCGAAGTTGTCAAACCTCA

EL6  
FIGURE 13

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EL6 protein sequence  
Molecular Weight 56687.90 Daltons  
500 Amino Acids  
59 Strongly Basic(+) Amino Acids (K,R)  
46 Strongly Acidic(-) Amino Acids (D,E)  
182 Hydrophobic Amino Acids (A,I,L,F,W,V)  
127 Polar Amino Acids (N,C,Q,S,T,Y)  
8.909 Isoelectric Point  
14.567 Charge at PH 7.0

SPTMPQAPMP	EFSSSVKLKY	VKLGYYQLVN	HFLSFLLIPI	MAIVAVELLR	MGPEEILNVW	NSLQFDLVQV
LCSSFFVIFI	STVYFMSKPR	TIYLVYDYSY	KPPVTCRVPF	ATFMEHSRLI	LKDKPKSVEF	QMRILERSGL
GEETCLPPAI	HYIPTPTMD	AARSEAQMI	FEAMDDLFFK	TGLKPKDVI	LIVNCSLFSP	TPSLSAMVIN
KYKLRSNIKS	FNLSGMGCSA	GLISVDLARD	LLQVHPNSNA	IIVSTEIITP	NYQGNERAM	LLPNCLFRMG
AAAIHMSNRR	SDRWRAKYKL	SHLVRTHRGA	DDKSFYCVYE	QEDKEGHVGI	NLSKDLMAIA	GEALKANITT
IGPLVLPASE	QLLFLTSLIG	RKIFNPKWKP	YIPDFKLAFE	HFCIHAGGRA	VIDELQKNLQ	LSGEHVEASR
MTLHRFGNTS	SSSLWYELSY	IESKGRMRRG	DRVWQIAFGS	GFKCNSAVWK	CNRTIKTPKD	GPWSDCIDRY
PVFIPEVVKL						

EL6  
FIGURE 14



[illegible]

EL7  
FIGURE 15

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EL7 protein sequence  
Molecular Weight 57848.80 Daltons  
516 Amino Acids  
59 Strongly Basic(+) Amino Acids (K,R)  
48 Strongly Acidic(-) Amino Acids (D,E)  
189 Hydrophobic Amino Acids (A,I,L,F,W,V)  
131 Polar Amino Acids (N,C,Q,S,T,Y)  
8.872 Isoelectric Point  
12.792 Charge at PH 7.0

MDGAGESRLG GDGGGDGSGV VQIROTRMLP DFLQSVNLKY VKLGYHYLIS NLLTLCLEPPL AVVISVEASQ  
MNPDDLKQLW IHLQYNLVS IICSAILVFG LTVYVMTRPR PVYLVDFSCY LPPDHLKAPY ARFMEHSRLT  
GDFDDSALEF QRKILERSGL GEDTYVPEAM HYVPPRISMA AAREEAEQVM FGALDNLFAN TNVKPKDIGI  
LVVNCSELFNP TPSLSAMIVN KYKLRGNIRS YNLGGMGCSA GVIAVDLAKD MLLVHRNTYA VVVSTENITQ  
NWYFGNKKSM LIPNCLFRVG GSAVLLSNKS RDKRRSKYRL VHVVRTHRGA DDKAFRCVYQ EQDDTGRTGV  
SLSKDLMAIA GETLKTNITT LGPLVLPIS QILFFMTLVV KKLFGNGVKP YIPDFKLAFE HFCIHAGGRA  
VIDELEKNLQ LSPVHVEASR MTLHRFGNTS SSSIWYELAY IEAKGRMRRG NRWVQIAFGS GFKCNSAIWE  
ALRHVKPSNN SPWEDCIDKY PVTLSY

EL7  
FIGURE 16

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/11384

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :AO1H 5/00, C07H 21/00; C12N 15/00, 15/82

US CL :800/205; 435/172.3; 536/23.6

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 800/205; 435/172.3; 536/23.6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
APS, DIALOG**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	WO 95/15387 A2 (CALGENE INC.) 08 June 1995, especially pages 57-71.	1,9,10,18-20,22-26, 28-30 ----- 2-8,11-17,21,27
X - Y	WO 96/13582 A2 (DNA PLANT TECHNOLOGY CORP.) 09 May 1996, especially pages 33-38.	1,9,10,18,19,24,25 ----- 2-8,11-17,20-23,26-30



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

07 AUGUST 1998

Date of mailing of the international search report

29 SEP 1998

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/11384

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	JAMES et al. Directed Tagging of the Arabidopsis FATTY ACID ELONGATION1 (FAE1) Gene with the Maize Transposon Activator. The Plant Cell. March 1995, Vol. 7, pages 309-319, see especially pages 316-317.	1,9,10 ----- 2-8,11,17-30